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Ronald L. Wilson, Director
Health Assessment Policy Staff
Office of Health Affairs (HFY-20)
Food and Drug Administration
5600 Fishers Lane, Room 15-22
Rockville, MD 20857

Re: BeneFIX™

FDA Docket No. 97E-0168

Dear Mr. Wilson:

Transmitted herewith is a copy of the application for patent term extension of U.S. Patent No. 5,171,569. The application was filed on March 6, 1997, under 35 U.S.C. § 156. In addition, a copy of a response, filed June 12, 1997, to the Notice of Informalities, mailed March 20, 1997, is also submitted herewith.

The patent claims a product that was subject to regulatory review under the Federal Food, Drug and Cosmetic Act. Subject to final review, the subject patent is considered to be eligible for patent term restoration. Thus, a determination by your office of the applicable regulatory review period is necessary. Accordingly, notice and a copy of the application are provided pursuant to 35 U.S.C. § 156(d)(2)(A).

Telephone inquiries regarding this matter should be directed to the undersigned at (703)306-3159.

Karin Tyson
Legal Advisor
Special Program Law Office
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects

cc: M.C. Meinert, Esq.
Genetics Institute, Inc.
Legal Affairs
87 Cambridge Park Drive
Cambridge, MA 02140

kt

#43

07/764073

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re	:	Patent Term Extension Application
Filed	:	March 6, 1997
For	:	USPN 5,171,569; Issued: December 15, 1992
For	:	FACTOR IX PREPARATIONS UNCONTAMINATED BY PLASMA COMPONENTS OR POX VIRUS

June 12 , 1997

VIA FACSIMILE (WITHOUT EXHIBITS)
VIA U.S. MAIL WITH EXHIBITS
(703) 308-6916
ATTN: Hiram H. Bernstein
Senior Legal Advisor
Special Program Law Office
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects
Assistant Secretary and Honorable
Commissioner of Patents and Trademarks
Box Patent Ext.
Washington, DC 20231

PETITION AND FEE FOR EXTENSION OF TIME UNDER 37 CFR §1.136

We hereby request a two month extension of time pursuant to 37 CFR §1.136(a) in which to respond to the Notice of Informalities mailed March 20, 1997. This extension resets the time for response from April 20, 1997, to June 20, 1997.

We authorize the charging of Three Hundred and Ninety dollars and of any additional fees and crediting of overpayment to our Deposit Account 07-1060, and for this purpose, we enclose a triplicate copy of this petition.

Respectfully submitted,

M. C. Meinert
Registration No. 31,544
Attorney for Applicant
Legal Affairs
Genetics Institute, Inc.
87 CambridgePark Drive
Cambridge, Massachusetts 02140-2387
617.498.8574

CERTIFICATE OF U.S. MAILING and FACSIMILE

Date of Deposit and Transmission 6.12.97

I hereby certify that the above-specified paper(s) and/or fee(s)are being deposited with the U.S. Postal Service under 37 CFR §1.10, in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Box Patent Ext, Washington, DC 20231, sent via facsimile (703) 308-6916 and U.S. Mail, to Hiram H. Bernstein,, on the date of deposit as indicated above.

n:\amend\factorix\amenext.2
June 12, 1997

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re	:	Patent Term Extension Application
Filed	:	March 6, 1997
For	:	USPN 5,171,569; Issued: December 15, 1992
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RESPONSE TO NOTICE OF INFORMALITIES AND ATTACHED EXHIBITS 1, 2, and 3

This is in response to the Notice of Informalities, received by telefacsimile, May 13, 1997 and to which a response is due June 13, 1997. The original of this Notice of Informalities appears to have been sent via regular mail on March 20, 1997 to the FDA; Applicant did not receive a copy of that dispatch. In the event that a response is due April 20, 1997 (rather than June 13, 1997), attached is a Petition requesting a two month extension of time under 37 C.F.R. §1.136, thereby extending the due date to June 20, 1997.

CERTIFICATE OF U.S. MAILING and FACSIMILE

Date of Deposit and Transmission 6.12.97

I hereby certify that the above-specified paper(s) and/or fee(s) are being deposited with the U.S. Postal Service under 37 CFR §1.10, in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Box Patent Ext., Washington, D.C., 20231, and via U.S. Mail and facsimile (703) 308-6916 to Hiram H. Bernstein, on the date of deposit as indicated above.

n:\amend\factorix\response.2
June 12, 1997

RESPONSE

Applicant acknowledges with thanks Ms. Karin Tyson's call to Applicant's undersigned attorney May 13 to inquire why a response to the Notice of Informalities had not yet been filed. Counsel for Applicant, M. C. Meinert, stated that the Notice of Informalities had never been received. Ms. Tyson kindly offered to send the notice via facsimile. This is in response thereto.

Compliance with 37 CFR §1.740(a)(9)

The PTO has requested further clarification that the factor IX protein of the approved product meets the conditions of claim 1 of USPN 5,171,569. Applicant's approved product meets all of the conditions of claim 1 as is set forth in the attached copy of "Chemical, Pharmaceutical and Biological Expert Report" from Volume 2, Part IC:1, pp. 1-19, of the Biological License Application (BLA) filed to obtain FDA marketing approval. (Exhibit 1)[1]

Specifically, claim 1 of the patent provides as follows:

> 1. A ***plasma-free*** preparation suitable for ***use in the treatment of*** human patients suffering from ***deficiency of factor IX***, said preparation comprising as active ingredient ***biologically active recombinant DNA-derived*** factor IX protein ***derived from a single human individual*** and which (1) ***essentially has the amino acid sequence of human factor IX*** **protein**, (2) is ***free from*** contamination by ***poxviruses*** and by all ***human plasma constituents***, and (3) has a ***specific activity*** defined as the concentration of test sample required to clot a given volume of factor IX-deficient plasma in a given time by the kaolin-cephalin method divided by the concentration of the factor IX protein in the test sample as determined by ELISA, ***of at least 90% of that of average normal human plasma.***

Applicant's factor IX product is plasma-free. In the production of rFIX, no materials, such as serum, serum extracts, or purified plasma proteins are used (Exhibit 1, page 5).

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[1]The Expert Report attached as Exhibit 1 is part of Applicant's Coagulation Factor IX (Recombinant) [rFIX], Biological License Application; Original Submission, 1996; Volume 1, pages 7-14.

Applicant's product is used in the treatment of human patients suffering from deficiency of factor IX as is set forth in the U.S. package insert. (Exhibit 2)

The active ingredient is biologically active recombinant DNA-derived factor IX protein. The protein is an expression product of recombinant DNA. (Exhibit 1)

The factor IX protein is encoded for by a DNA sequence from a single human individual having the Ala 148 allelic form[2] of the factor IX molecule (Exhibit 1, page 1), and which:

(1) essentially has the amino acid sequence of human factor IX protein (Exhibit 1, page 1)

(2) is free from contamination by poxviruses and by all human plasma constituents (Exhibit 1, pages 1, 3, 5), and

(3) has a specific activity, expressed as International Units activity/mg protein, of at least 90% of that of average normal human plasma.[3] (Exhibit 1, pages 1, 3)

Accordingly, Applicant's approved product meets all of the limitations of claim 1 of USPN 5,171,569.

---

[2]There is a polymorphism in plasma factor IX; in some individuals amino acid 148 is threonine but in others it is alanine.

[3]Specific activity has the units: International Units actvity per milligram of protein = IU/mg. One International Unit of factor IX is equal to .005 mg. Hematology, Basics Principles & Practice, Eds. Hoffman et al. Churchill Livingstone, Pubs. (1991). (Exhibit 3, p. 1217).

Average normal human plasma has a factor IX specific activity of 1/.005 mg = 250 IU/ mg. Ninety percent of 250 IU/mg = 225 IU/mg.

Applicant's factor IX has a specific activity of greater than 260 IU/mg (Exhibit 1, page 1), which is greater than 90% of the specific activity of average normal human plasma.

Clarification of Particular Federal Statutes Under Which Regulatory Review Occurred

The applicable federal statutes are §351 of the Public Health Service Act and §505 of the Federal Food Drug and Cosmetic Act; regulatory review is under 21 CFR §601.

CONCLUSION

Applicant believes that the Application for the Extension of Patent Term is now in conformity and Applicant respectfully requests that the Application for Extension of Patent Term under 35 U.S.C. §156 be granted.

Should the PTO believe that a telephonic interview would assist in clarifying any remaining issues, or to otherwise expedite the application, Applicant respectfully invites the PTO to call the undersigned attorney at the telephone number provided below.

Applicant hereby authorizes any payment which may be due with regard to this paper from Deposit Account No. 07-1060.

Respectfully submitted,

M. C. Meinert
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From textbook.

HEMATOLOGY

Basic Principles and Practice

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The Molecular Basis of Blood Coagulation

93

Bruce Furie and Barbara C. Furie

INTRODUCTION

Blood coagulation is a host defense system that maintains the integrity of the high-pressure closed circulatory system. After tissue injury, alterations in the capillary bed and laceration of venules and arterioles lead to extravasation of blood into soft tissues or external bleeding. To prevent excessive blood loss, the hemostatic system, which includes platelets, vascular endothelial cells, and plasma coagulation proteins, is called into play. Immediately following tissue injury, a platelet plug is formed through the processes of platelet adhesion and aggregation. Blood coagulation may be considered a mechanism for rapid replacement of an unstable platelet plug with a chemically stable fibrin clot. A series of interdependent, enzyme-mediated reactions translate the molecular signals that initiate blood coagulation into a major biologic event, the formation of the fibrin clot.

The generation of thrombin and the formation of a fibrin clot have been thought to propagate through two separate pathways, the intrinsic pathway and the extrinsic pathway.[1,2] To generate a clot via the intrinsic pathway, components *intrinsic* to whole blood are required. To generate a clot via the extrinsic pathway, components intrinsic to whole blood are required along with an

Table 93-1. Properties of the Genes, mRNAs, and Gene Products of the Components of the Blood Coagulation Cascade

	Molecular Weight	Gene (kb)	Chromosome[a]	mRNA	Exons (kb)	Plasma Concentration (μg/ml)	Function
Prothrombin	72,000	21	11p11-q12	2.1	14	100	Protease zymogen
Factor X	56,000	22	13q34	1.5	8	10	Protease zymogen
Factor IX	56,000	34	q26-27.3	2.8	8	5	Protease zymogen
Factor VII	50,000	13	13q34	2.4	8	0.5	Protease zymogen
Factor VIII	330,000	185	q28	9.0	26	0.1	Cofactor
Factor V	330,000			7.0		10	Cofactor
Factor XI	160,000	23			15	5	Protease zymogen
Factor XII	80,000	12	5	2.4	14	30	Protease zymogen
Fibrinogen	340,000					3000	Structural
Aα chain	66,000		4q26-q28		5	—	
Bβ chain	52,000		4q26-q28		8	—	
γ chain	46,000		4q26-q28		9	—	
Protein C	62,000	11		1.8	8	4	Protease zymogen
Protein S	80,000			2.4		25	Cofactor
vWF	225,000 × n	175	12pter-p12	8.5	52	10	Adhesion
Tissue Factor	37,000	12	1pter-p12	2.1	6	0.0	Cofactor/initiator

Abbreviation: n, number of subunits, where the subunit M_r is 225,000.
[a] Chromosomal assignments from Royle et al.[71]
(Adapted from Furie and Furie,[198] with permission.)

activating substance, *tissue factor*, which is *extrinsic* to blood (Table 93-1). Tissue factor, also known as *tissue thromboplastin*, is a cell surface protein that is expressed upon cellular injury. The intrinsic pathway of blood coagulation includes protein cofactors and enzymes (Fig. 93-1). This pathway is initiated by the activation of factor XII by kallikrein on negatively charged surfaces, including glass in vitro. High molecular weight kininogen facilitates this activation. The enzyme form of factor XII, factor XIIa, catalyzes the conversion of factor XI, a proenzyme, to its active enzyme form, factor XIa. In the presence of calcium ions factor XIa activates the proenzyme factor IX to its enzyme form, factor IXa. Although the details of the reaction are incompletely known, factor IXa appears to bind to the cofactor factor VIIIa bound on membrane surfaces in the presence of calcium ions to generate a complex with enzyme activity known as *tenase*. This tenase complex converts the proenzyme factor X to its enzyme form, factor Xa. In a parallel series of interactions factor Xa binds to the cofactor factor Va, bound on membrane surfaces, in the presence of calcium ions to generate a complex with enzyme activity known as *prothrombinase*. This complex converts the proenzyme, prothrombin, to its enzyme form, thrombin. Thrombin acts on fibrinogen to generate the fibrin monomer, which rapidly polymerizes to form the fibrin clot. During laboratory analysis of blood clotting, the intrinsic pathway of blood coagulation is evaluated by using the activated partial thromboplastin time (PTT). The clotting of plasma is initiated by the addition of negatively charged particles such as kaolin.

The extrinsic pathway of blood coagulation also includes protein cofactors and enzymes (Fig. 93-1). This pathway is initiated by the formation of a complex between tissue factor on cell surfaces and factor VII. This complex, like the tenase complex, converts factor X to its active form, factor Xa, which binds to the cofactor factor V, bound on membrane surfaces in the presence of calcium ions, to generate the prothrombinase complex. This complex converts prothrombin to thrombin, which converts fibrinogen to fibrin to generate the fibrin clot. During laboratory analysis of blood clotting, the extrinsic pathway of blood coagulation is evaluated by using the prothrombin time. The clotting of plasma is initiated by the addition of exogenous tissue factor.

STRUCTURE OF THE BLOOD COAGULATION PROTEINS

Domain Structure

The blood coagulation proteins each contain multiple functional units, which are derived from common ancestral genes (Fig. 93-2). As a rule these functional units, or domains, are encoded by individual exons. Although the domains are not structurally identical, they have sufficient homology in structure to suggest homology of function as well. These domains are responsible for directing protein trafficking and post-translational processing during biosynthesis and for membrane binding properties, protein complex formation, and enzyme function of the mature protein.

Signal Peptide

The nascent chains of secreted proteins contain a short domain that allows translocation of the growing polypeptide chain into the endoplasmic reticulum. This domain is dominated by hydrophobic amino acids and is usually about 15 to 30 residues in length. The blood coagulation proteins found in the plasma are initially synthesized with a signal peptide, which is cleaved within the endoplasmic reticulum.

Propeptide/γ-Carboxyglutamic Acid-Rich Domain

The vitamin K-dependent proteins contain a propeptide domain between the signal peptide and the γ-carboxyglutamic acid-rich N-terminal region. This propeptide, which is known to be 18 residues long in factor IX[3,4] and 24 residues long in protein C,[5] contains a γ-carboxylation recognition site, which directs γ-carboxylation of the vitamin K-dependent proteins after synthesis.[6] Adjacent to the carboxylation recognition site is the γ-carboxyglutamic acid-rich region of these proteins. Glutamic acid residues within this region serve as substrates for the vitamin K-dependent γ-carboxylase. After carboxylation, the propeptide is cleaved from the mature protein in a late post-translational processing step. A single exon in the vitamin K-dependent proteins encodes the propeptide (including the γ-carboxylation recogni-

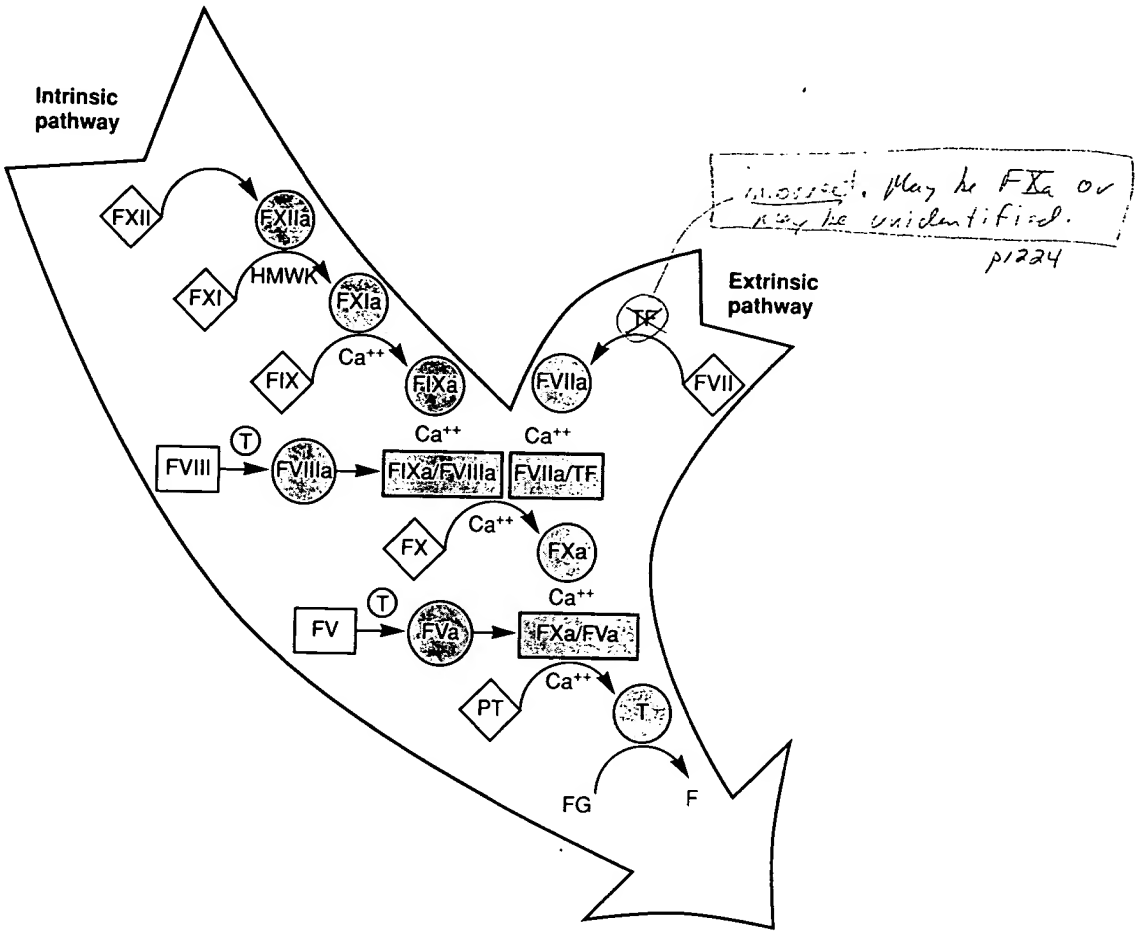

Fig. 93-1. The blood coagulation cascade. Glycoprotein components of the intrinsic pathway include factors XII, XI, IX, VIII, X, V, prothrombin, and fibrinogen. Glycoprotein components of the extrinsic pathway, initiated by the action of tissue factor located on cell surfaces, include factors VII, X, V, prothrombin, and fibrinogen. The cascade reactions culminate in the conversion of fibrinogen to fibrin and the formation of a fibrin clot. Certain reactions, including the activation of factor X and prothrombin, take place on membrane surfaces. Proenzymes, diamonds; procofactors, squares; enzymes and cofactors, circles; macromolecular complexes on membrane surfaces, shaded rectangles; FG, fibrinogen; F, fibrin; PT, prothrombin; T, thrombin; HMWK, high molecular weight kininogen.) (Modified from Furie and Furie,[198] with permission.)

tion site) and the γ-carboxyglutamic acid-rich region of approximately 40 residues. The γ-carboxyglutamic acid domain of the vitamin K-dependent proteins contains 10 to 12 γ-carboxyglutamic acid residues and is critical to the calcium ion-binding properties of these proteins. This domain is responsible for promoting the calcium-dependent interaction of these proteins with membrane surfaces, a characteristic essential for the function of these proteins.

The three-dimensional structure of the γ-carboxyglutamic acid-rich region in bovine prothrombin is disordered in the absence of calcium ions[7] but contains considerable α-helix and β-pleated sheet when the structure is stabilized by calcium ions[8] (Fig. 93-3). Because the calcium ions have not been identified in the crystal structure, the structures of the metal binding sites are unknown. Furthermore, the mechanism by which this region promotes interaction of the protein with membrane surfaces remains to be elucidated.

Epidermal Growth Factor Domain

The epidermal growth factor (EGF)-like domain is a common motif found in many proteins, including some of the proteins involved in blood coagulation; in the blood clotting proteins it is highly homologous with the EGF precursor.[9] This domain, about 43 to 50 amino acid residues in length, contains three disulfide bonds arranged in a characteristic covalent structure. Although the EGF domain in some proteins mediates their interaction with an EGF or EGF-like receptor on cell surfaces, the function of the EGF domains on the blood clotting proteins remains uncertain. These regions may mediate the binding of factor IX to endothelial cells.[10] Factors IX, X, and VII contain two adjacent EGF domains (as does protein C, while protein S has four EGF domains). Factor XII contains two nonadjacent EGF domains, while tissue plasminogen activator and prourokinase each have a single EGF domain.

Kringle Domain

Another common motif in proteins is the kringle domain. This region, with a characteristic covalent structure also defined by a pattern of three disulfide bonds, is about 100 amino acid residues in length. The three-dimensional structure of the prothrombin kringles reveals an oblate ellipsoid, with folding defined by close contacts between the sulfur atoms of two of the disulfide bridges.[7] Internal structures are well conserved among kringles from various proteins, but molecular surface differences must relate to differences in function. It is likely that kringle domains play a role in protein complex formation. These structures appear in prothrombin,[11] factor XII,[12] plasminogen,[13] prourokinase,[14] and tissue plasminogen activator[15] among the proteins involved in hemostasis.

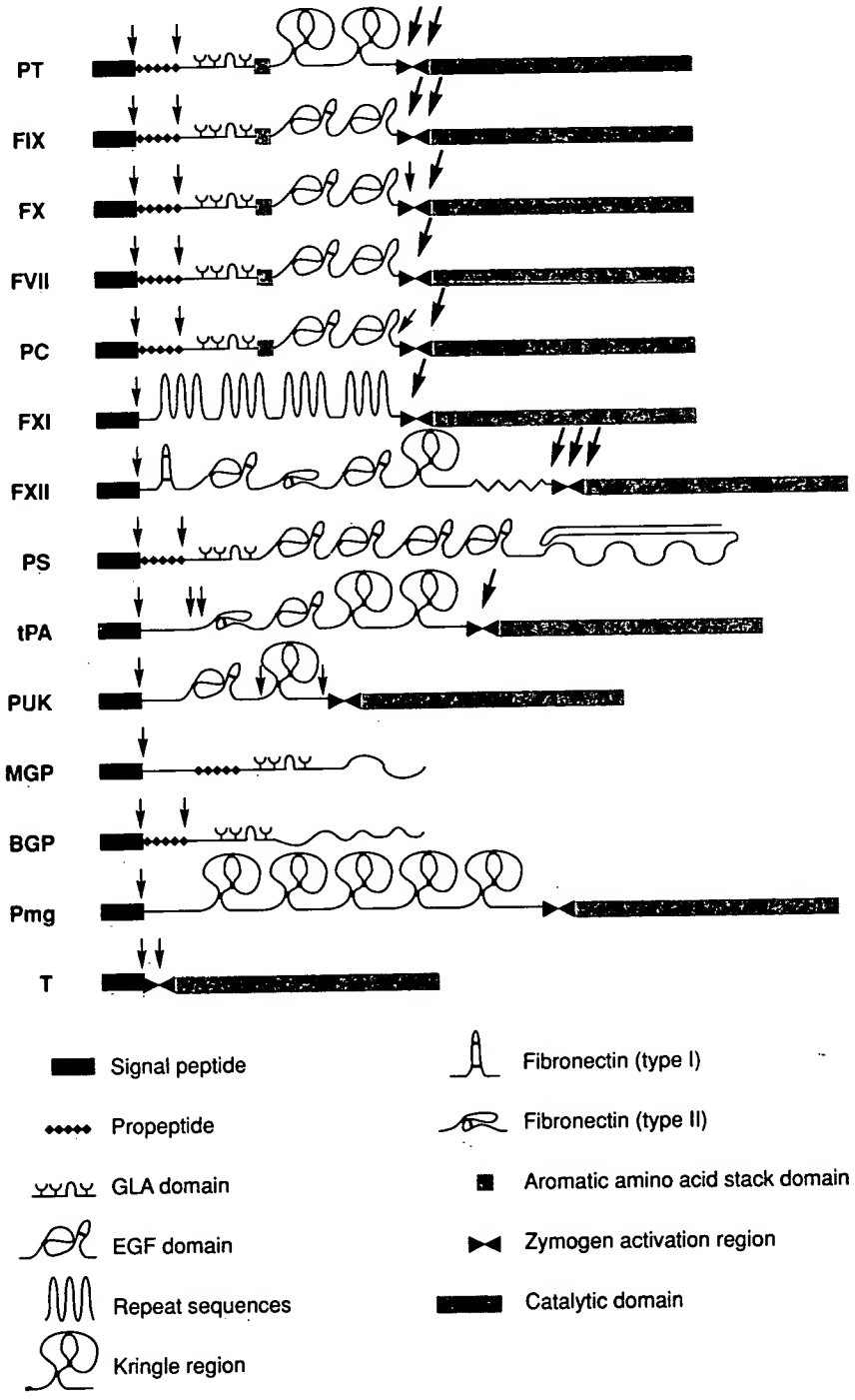

Fig. 93-2. Structural domains of the proteins involved in hemostasis and related proteins. Domains, identified in the key, include the signal peptide, the propeptide, the Gla domain, the epidermal growth factor (EGF) domain, the repeat sequences, the kringle region, the fibronectin (type I and II) domains, the aromatic amino acid stack, the zymogen activation region, and the catalytic domain. The sites of proteolytic cleavage associated with synthesis of the mature protein are indicated by the thin arrows and those associated with zymogen activation by the thick arrows. PT, prothrombin[199] ; FIX, factor IX[200,201]; FX, factor X[46,57]; FVII, factor VII[62]; PC, protein C[202,203]; FXI, factor XI[24]; FXII, factor XII[12,17]; PS, Protein S[204]; tPA, tissue-type plasminogen activator[15]; PUK, prourokinase[14]; MGP, matrix Gla protein[205]; BGP, bone Gla protein[127]; Pmg, plasminogen[13]; T, trypsin. (Modified from Furie and Furie,[198] with permission.)

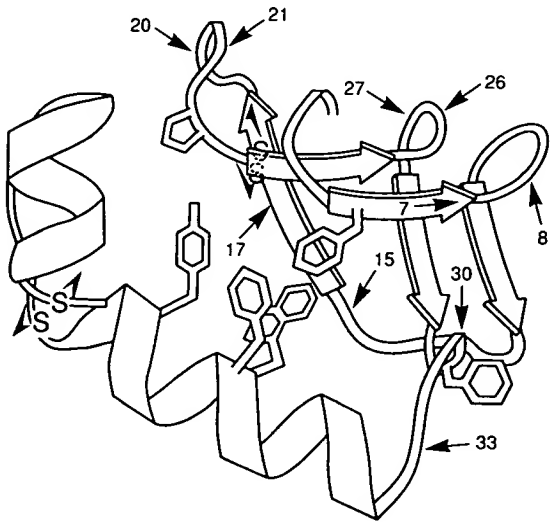

Fig. 93-3. Three-dimensional structure of the γ-carboxyglutamic acid-rich region of prothrombin. This region is responsible for metal binding and membrane-protein interaction. (Modified from Soriano-Garcia et al,[8] with permission.)

Catalytic Domain

A catalytic domain, highly homologous with the structure of trypsin and chymotrypsin, is common to all the blood clotting enzymes. This domain includes a site for the conversion of an inactive proenzyme to an active enzyme via cleavage of peptide bonds, a process known as *zymogen activation*. Furthermore, this domain contains the enzymatic machinery for cleavage of peptide bonds, the specific recognition site for macromolecular substrates, and the site for interaction with specific protein inhibitors that regulate enzyme activity. The blood clotting proteases are serine proteases, a class of enzymes with a common mechanism of enzyme action that include a serine, aspartic acid, and histidine within the active site. The catalytic domain of the blood clotting proteases have an active site and an internal core that is nearly identical to that of trypsin.[16] The molecular surfaces surrounding the enzyme active site are likely responsible for defining the extended substrate binding site of the enzyme. Indeed, solution of the crystal structure of human α-thrombin has emphasized the structural homology between thrombin and other well-characterized serine proteases while simultaneously providing details of the molecular structure that are not available from models derived indirectly from other homologous structures.[17]

Other Domains

Other motifs that appear within the proteins involved in hemostasis include the aromatic amino acid stack domain, the repeat regions observed in factor XI and, the fibronectin type I and type II domains. The functions of these domains are unknown.

Components of the Intrinsic and Extrinsic Pathways

Factor XII

The factor XII gene, located on chromosome 5, contains 14 exons and 13 introns[12,18] (Fig. 93-4). Exon I encodes the signal peptide; exon II encodes a segment with no structural homology with other proteins; exons III and IV code for a region homologous with the type II fibronectin structure; exon V and VII each encode EGF domains; exon VI encodes a fibronectin finger domain that intervenes between the EGF domains; and exons VIII and IX each encode a kringle domain. The trypsin-like catalytic domain, including the activation region, is encoded by the remaining exons X to XIV. The gene organization of the catalytic domains parallels that of urokinase, factor XI, and tissue plasminogen activator. The factor XII messenger RNA is 2.4 kb in length.[18,19] The mature, plasma form of factor XII is composed of 596 amino acids residues in a single polypeptide chain.[12,19]

Factor XII, also known as *Hageman factor*, is the first component of the intrinsic pathway. As such, it is a component of the contact phase of activation of blood coagulation that is observed in vitro. This protein does not appear to have a physiologic role in blood coagulation in vivo since patients lacking this protein do not have a bleeding disorder. The protein circulates in the blood as a single chain proenzyme of 80,000 molecular weight.[20,21] Factor XII is a glycoprotein, with carbohydrate attached to Asn 230 and Asn 414. Other glycosylation sites include Thr 280, 286, 309, 310, and 318 and Ser 289. The surface binding properties of factor XII, specifically the enhancement of the rate of factor XII activation by negatively charged surfaces, may be mediated by the positively charged amino acid sequence His-Lys-Tyr-Lys, a structure common to factor XII and kininogen.[22] The factor XII concentration in plasma is about 30 μg/ml. Factor XII has a plasma half-life of 2 days.[23]

Factor XI

The gene for factor XI, an intrinsic pathway component, contains 15 exons and 14 intervening sequences[24] (Fig. 93-4). Exon I encodes a 5′ untranslated sequence, while exon 2 encodes a signal peptide. Four repeat sequences are each encoded by two exons: repeat 1 by exons III and IV, repeat 2 by exons V and VI, repeat 3 by exons VII and VIII, and repeat 4 by exons IX and X. The trypsin-like domain, responsible for the proteolytic function of the enzyme form of the zymogen, is encoded by exons XII to XV. Factor XI has a 160,000 molecular weight[25] and is composed of two identical chains bound together by disulfide bonds.[26] Factor XI circulates in the blood at a concentration of 5 μg/ml. Its biologic half-life is about 3 days.[27,28]

Factor IX

Factor IX plays a critical role in blood coagulation (Ch. 102). Its gene, adjacent to the factor VIII gene, is located on the X chromosome. Defects in this gene, both major and minor, are the cause of hemophilia B (Ch. 103). This gene is 34 kb in length, contains eight exons,[29] and has a structure that is highly homologous with the structures of the factor VII, factor X, and protein C genes (Fig. 93-4). These genes are sufficiently similar to suggest that they were derived ancestrally from a common related gene. Factor IX is encoded on a 2.8-kb mRNA transcript. The mature protein, with a molecular weight of 56,000, requires vitamin K for its synthesis and contains 12 γ-carboxyglutamic acid residues. The fully carboxylated form of the protein binds to metal ions and to membrane surfaces in the presence of metal ions. Factor IX contains two classes of metal-binding sites, which are defined by γ-carboxyglutamic acid,[30] and a third class, which is a component of the EGF domain.[31,32] The site within the EGF domain appears to be defined by aspartic acid 64, a residue that is partially β-hydroxylated. Factor IX and factor IXa bind to phospholipid membranes composed of phosphatidylserine-phosphatidycholine.[33,34] This interaction requires both calcium ions and fully carboxylated factor IX.[34] The expression of phospholipid-binding properties involves two metal-dependent conformational transitions.[34] Factors IX and IXa bind to activated platelets but not to resting platelets.[35,36] However, it is not known whether a receptor is present on the platelet surface or what role surface phospholipid plays in factor IX binding. In contrast, Gla-domainless factor IX binds to endothelial cells. A factor IX receptor on endothelial cells has been tentatively identified.[37]

The factor IX concentration in plasma is about 5 μg/ml. This

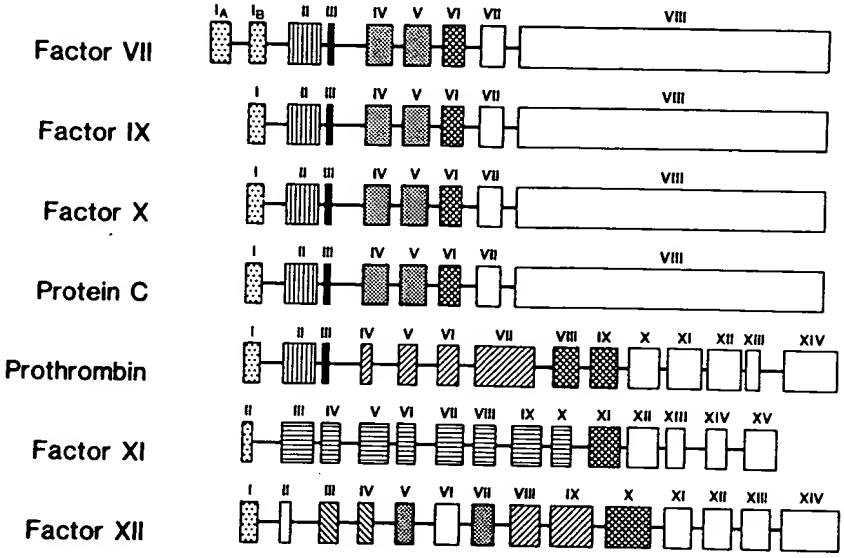

Fig. 93-4. Gene structures among the blood coagulation serine proteases. Exon, are shown schematically according to scale. Introns, thick lines, are not drawn to scale. The exons code for most of the individual domains are shown in the key for Fig. 93-2: PT, prothrombin[72]; FIX, factor IX[29]; FX, factor X[44,46]; FVII, factor VII[59]; PC, protein C[5,202]; FXI, factor XI[24]; FXII, factor XII.[12,17] (Modified from Furie and Furie,[198] with permission.)

protein has a plasma half-life of about 24 hours.[38] As with other proteins of this molecular size, it partitions between the intravascular and extravascular space. A comprehensive review of the structure of the factor IX gene and the protein that it encodes is presented in Chapter 102.

Factor VIII

Factor VIII is a critical cofactor that is required for normal blood clotting (see Ch. 98). Defects in the factor VIII gene that lead to the deficiency of factor VIII are the cause of hemophilia A (see Ch. 99). The factor VIII gene is 186 kb in length and is divided into 26 exons[39,40] (Fig. 98-1). As such, it is one of the largest genes yet discovered. Located on the X chromosome, it is near the locus of the factor IX gene. Factor VIII is synthesized as a single polypeptide chain including a 19-residue signal peptide and in its mature form contains 2,332 amino acid residues.[39,40] Although this factor is synthesized by many cell types, the liver appears to be the primary site of synthesis.[41] A molecular weight of 330,000 has been estimated for the glycosylated protein. The protein sequence of factor VIII demonstrates marked sequence homology with factor V, another protein cofactor.[42] Factor VIII circulates in the blood as a heterodimer composed of two polypeptide chains derived from the original single chain. These two chains, including one of relative molecular weight (M_r) 80,000 and one of M_r varying between 90,000 and 200,000, are derived from the carboxy terminus and the amino terminus of the single chain precursor, respectively. Their interaction is calcium ion-dependent. Factor VIII in its circulating form is inactive or minimally active as a cofactor in blood coagulation. It circulates in the blood at very low concentration (100 ng/ml) bound to von Willebrand factor (vWF). Its plasma half-life is about 8 to 12 hours.[43] Factor VIII is converted into its active cofactor form by the proteolytic cleavage of two or more peptide bonds within the protein by thrombin. The structure and function of Factor VIII are described in detail in Chapter 98.

Factor X

The factor X gene, located on the long arm of chromosome 13 adjacent to the factor VII gene, is composed of eight exons and is 22 kb in length[44] (Fig. 93-4). The organization of this gene is identical to that of the factor IX gene. Exon I encodes the signal peptide, exon II the propeptide/γ-carboxyglutamic acid-rich domain, exon III the short aromatic amino acid stack domain, exons IV and V the two EGF domains, exon VI the activation region, and exons VII and VIII the catalytic domain. After transcription, the factor X mRNA of about 1.5 kb includes a short 3′ untranslated region following the stop codon.[45,46] The polyadenylation signal is located within the 3′ end of the coding sequence.

Factor X, with a molecular weight of 56,000 is synthesized as a single polypeptide chain.[44,46] However, factor X as isolated from plasma is composed of two polypeptide chains, a heavy chain with a 38,000 molecular weight[47–49] and a light chain with an 18,000 molecular weight.[50,51] These chains are linked by a single disulfide bond. The Arg-Lys-Arg sequence at residues 139 to 141 in the single polypeptide chain appears very susceptible to intracellular or extracellular proteolysis; thus yielding the predominant two-chain form.[44,46] The light chain of factor X includes 11 γ-carboxyglutamic acid residues at positions 6, 7, 14, 16, 19, 20, 25, 26, 29, 32, and 39 in the human protein.[52] A single β-hydroxyaspartic acid residue is located in the first EGF domain at residue 63. Bovine factor X contains both asparagine-linked carbohydrate at asparagine 36 and threonine-linked carbohydrate at threonine 300.[53] Like prothrombin, the asparagine-linked sugars, contain NeuAcα2 → 3Galβ1 → 3(NeuAcα2 → 6)GlcNAc in the outer chain.

Factor X is a calcium-binding protein which interacts with membrane surfaces in the presence of calcium. It contains both low- and high-affinity metal-binding sites,[54] occupancy of which leads to conformational changes and the expression of membrane-binding properties.[55] Like the other vitamin K-dependent proteins, factor X binds preferentially to acidic phospholipid surfaces.[56–58] Bound factor X is an "extrinsic" membrane protein in that no component of it is embedded within the membrane.

The plasma concentration of factor X is maintained at about 10 μg/ml. Its half-life in plasma is about 36 hours.[38] Coagulopoietin X, an activity that has been identified in plasma rendered deficient in factor X, may play a role in the regulation of the plasma concentration of this factor.[59] However, as with the other plasma clotting proteins, the molecular basis of the control of factor X plasma levels is not known.

Factor VII

The factor VII gene is 13 kb in length[60] and is located on the long arm of chromosome 13, immediately adjacent to the factor X gene.[61] The coding region is found on nine separate exons (Fig.

93-4). Pre-profactor VII is synthesized via two alternate forms. In one form, incorporating exon IB encoding for the signal peptide, factor VII arises from a gene whose gene organization is identical to the factor IX gene. In a second form, exon IA directs the coding of the signal peptide instead of exon IB. In this form, the pre-profactor VII has a polypeptide extension of the NH_2 terminus that elongates the signal peptide/propeptide from 38 to 60 residues. Exon II encodes the propeptide and γ-carboxyglutamic acid-rich domain, and exon III encodes the short aromatic amino acid stack domain, a segment common to all the vitamin K-dependent proteins. Exons IV and V each encode one of the EGF domains. The catalytic domain is coded by exons VI, VII and VIII, with the activation peptide encoded within exon VI. The mRNA for factor VII is about 2.4 kb in length,[62] with a 3′ untranslated region of 1.0-kb length and a poly(A) tail located after the stop codon.

Factor VII is a component of the extrinsic pathway of blood coagulation and forms a complex with tissue factor to generate an enzyme complex that activates factor X. Human factor VII, with a molecular weight of 50,000, circulates in plasma as a single-chain zymogen containing 406 amino acid residues.[62] The amino-terminal domain includes 11 γ-carboxyglutamic acid residues at positions 6, 7, 14, 16, 19, 20, 25, 26, 29, 34, and 35 in bovine factor VII.[63] Aspartic acid 63 is partially β-hydroxylated. Factor VII is a glycoprotein, containing 13 percent carbohydrate.

In contrast to other proenzymes involved in blood coagulation, factor VII preparations have low but significant levels of enzyme activity.[64] This characteristic may be an important feature for initiation of the extrinsic pathway, allowing generation of small amounts of factor Xa, which can activate factor VII. This model would allow significant amplication of thrombin formation via the extrinsic pathway. However, more recent information suggests that factor VII may be a true zymogen, with no enzymatic activity prior to cleavage.[65]

Factor V

Factor V is a plasma glycoprotein with a molecular weight of 330,000. This protein is a critical cofactor, which in its activated form facilitates activation of prothrombin by factor Xa. Factor V is a single-chain protein, which circulates in the blood in a precursor, inactive cofactor form. The factor V gene, which has not been described, encodes a 7-kb mRNA, which itself encodes a pre-factor V including a 28-amino acid residue signal peptide and a mature protein composed of 2,196 amino acids residues.[66,67] The heavy chain region is composed of two domains with notable structural homology, termed the A domain. The light chain region is composed of another A domain and two homologous C domains. The heavy chain region and light chain region are joined by a connecting region known as the B domain. The A1-A2-B-A3-C1-C2 domain structure is also present in factor VIII.[42]

Although the liver appears to be the primary site of synthesis of factor V, megakaryocytes also synthesize this protein. In addition to its presence in plasma, factor V is a component of the α-granules in megakaryocytes and subsequently in platelets[68] and is secreted upon platelet stimulation with specific agonists. Factors V and Va bind to two classes of binding sites on the surface of platelets.[69] However, the higher-affinity binding sites interact specifically with factor Va and not with factor V.

The plasma concentration of factor V is 10 μg/ml, and its plasma half-life is about 12 hours.[70]

Prothrombin

The prothrombin gene, located on chromosome 11,[71] is 21 kb in length, and is composed of 14 exons, each encoding all or part of a functional domain of prothrombin[72] (Fig. 93-4): signal peptide (exon I), propeptide and γ-carboxyglutamic acid-rich domain (exon II), aromatic amino acid stack domain (exon III), two kringle domains (exons IV to VII), the activation region (exons VIII and IX) and the catalytic domain (exons X to XIV). The introns vary considerably in size, from 84 bp for the intron between exons VIII and IX to 9,447 bp for that between exons XII and XIII. Although the structure of prothrombin is homologous with those of factor IX, factor X, factor VII and protein C, the prothrombin gene demonstrates only partial homology with the genes of these proteins. Exons I through III are shared by all these proteins, but prothrombin contains exons IV to VII encoding the kringle domains, and has a homologous serine protease domain composed of seven exons, in contrast to the two exons found in the factor IX gene family. The prothrombin mRNA is 2.1 kb in length and includes a 5′ untranslated region greater than 150 bp, a 1.8-kb open reading frame, and a 97-bp 3′ untranslated region. Mechanisms of transcriptional and translational regulation have not been studied.

Prothrombin is a plasma glycoprotein with a molecular weight of 72,000.[11,73,74] On the basis of direct protein sequence analysis and the predicted sequence based upon the nucleotide sequence of the cDNA, the complete amino acid sequence of the human protein is known.[72–74] As with all the blood clotting proteins, prothrombin is synthesized with a hydrophobic signal peptide from residues −43 to −19. After translocation to the rough endoplasmic reticulum, the signal peptide is removed by a signal peptidase. The propeptide, containing the γ-carboxylation recognition site,[6] includes residues −18 to −1. During protein synthesis but after γ-carboxylation, this peptide is removed by an intracellular propeptidase. The mature prothrombin that circulates in the plasma is composed of 579 amino acid residues arranged in a single polypeptide chain. The 10 γ-carboxyglutamic acid residues are located in the Gla domain of human prothrombin at residues 6, 7, 14, 16, 19, 20, 25, 26, 29, and 32. Carbohydrate represents about 10 percent of the mass of prothrombin. *N*-Asparagine-linked carboyhydrate is attached to asparagine 78, asparagine 100, and asparagine 373 in bovine prothrombin,[75] and human prothrombin likely contains carbohydrate at the homologous amino acids. Complex asparagine-linked oligosaccharides include NeuAcα2 → 3Galβ1 → 3(NeuAcα2 → 6)GlcNAc. The short aromatic amino acid stack domain has significant α-helical structure and serves to link the G1a domain to two kringle domains,[7] which are similar to structures found in factor XII, plasminogen, and tissue plasminogen activator and are defined structurally by the pattern of disulfide bonds. The function of these domains is uncertain, but they may be important for protein complex formation with factor Va. The remainder of prothrombin, accounting for approximately half of the protein structure, represents the catalytic domain. This region includes the activation domain that is critical for the conversion of the zymogen into the active enzyme and the trypsin-like region that possesses the protease activity. Prothrombin has no coagulant activity in its zymogen form and must be converted to thrombin in order to participate in blood coagulation.

The metal-binding properties of prothrombin are conferred by γ-carboxyglutamic acid residues.[78] Abnormal (des-γ-carboxy)prothrombin, lacking γ-carboxyglutamic acid, does not bind to calcium ions and does not interact with membrane surfaces in the presence of calcium.[77,78] Prothrombin binds calcium and other metal ions via two classes of metal binding sites,[79–82] and upon metal binding, it undergoes conformational changes leading to expression of membrane-binding properties.[83–85] Concommitantly, neoantigens are exposed on the metal-stabilized conformers of prothrombin.[86–89] Prothrombin is an extrinsic membrane-binding protein. In the presence of calcium ions, a surface of the prothrombin-metal complex interacts with phospholipid vesicles; a marked preference for acidic phospholipids, specifically phosphatidylserine, has been demonstrated. The N-terminal third of the protein contains the lipid-binding domain.

The plasma concentration of prothrombin is about 100 μg/ml,[90,91] and the plasma half-life of prothrombin is about 3 days.[38,92]

Part IC EXPERT REPORTS

IC:1 Chemical, Pharmaceutical, and Biological Expert Report

IC:1.1 Product Profile

a) Type of application: a new active substance

b) Chemical and pharmacokinetic properties

Chemical

BeneFIX™, Coagulation Factor IX (Recombinant) [rFIX; also referred to as rhFIX] is a 415-amino acid glycoprotein (approximately 55 kDa) that is produced in Chinese hamster ovary (CHO) cells (FIX.1F cell line) and subsequently very highly purified using a four-step chromatography process. For maximum assurance of safety, the purification process also contains a viral filtration step capable of removing viruses. Other than use of the FIX.1F cell line and recombinant human insulin made in *E.coli*, no animal or human proteins are used in the manufacture and purification of rFIX. The rFIX drug product is a lyophilized powder for injection cake produced from a solution containing approximately 100 IU rFIX/mL, 10 mM L-histidine, 0.26 M glycine, 1% sucrose, and 0.005% polysorbate 80, pH 6.8. The lyophilized formulation is stable, contains no preservatives, and contains no added animal or human raw materials. The drug product is available in 1000-, 500-, and 250-IU dosage strengths which are approximately 100-, 100-, and 50-IU rFIX/mL, respectively, when reconstituted with Sterile Water for Injection as directed.

The amino acid sequence of rFIX is identical to that of the Ala 148 allelic form of plasma-derived FIX (pdFIX). Total impurities account for ≤1%, as determined by SDS-PAGE. The specific activity of rFIX, as determined by the traditional one-step clotting assay, is approximately 260 IU/mg.

With respect to post-translational modifications, rFIX contains approximately 11.5 γ-carboxyglutamyl (Gla) residues, with full occupancy of the Gla residues important for calcium binding. rFIX is glycosylated via both N- and O-linkages. The N-linked oligosaccharides of rFIX are of the complex type, with structures ranging from tetrasialylated to neutral. Partial β-hydroxylation of Asp^{64} is inferred from peptide mapping and mass spectrometric analysis. Partial sulfation of rFIX occurs at Tyr^{155}. The amino acid residues of rFIX are not oxidized or phosphorylated, whereas a low degree of deamidation may be present.

Pharmacokinetic Parameters and Clinical Efficacy

Animal. Pharmacology studies of rFIX in a canine model of hemophilia B indicate that rFIX can correct genetically deficient hemostasis. Evaluation of rFIX in the modified Wessler Stasis model in rabbits has shown rFIX to have very low thrombogenicity relative to commercially available forms of human plasma-derived FIX (pdFIX). Pharmacokinetic parameters of rFIX have been determined after single and multiple intravenous doses in Sprague-Dawley rats, beagle dogs, and mixed-breed

hemophilia B dogs. The PK parameter estimates were linear across all doses evaluated (40 to 200 IU/kg), and accumulation of rFIX was not found. The PK parameters obtained in the studies in dogs and rats that compared rFIX to pdFIX (Mononine®, Armour Pharmaceuticals, Inc.) are similar to those obtained in human studies (see below). In studies investigating how structural features of rFIX and pdFIX affect recovery, it was determined that charged moieties on the activation peptide contribute to the modulation of recovery within the range observed for rFIX and pdFIX.

Human. In a crossover pharmacokinetic evaluation of rFIX and Mononine® in previously treated patients (n=11), infusion of rFIX into patients with hemophilia B resulted inin vivo recoveries of approximately 38 ± 14%. The plasma half-life of factor IX was 18.1 ± 5.1 hours. The observed 0.84 IU/dL rise in rFIX activity per IU/kg of rFIX administered is within the range outlined in the core Summary of Product Characteristics (SmPC) for human coagulation IX concentrates in the European Community (EC) *Note for Guidance* III/3458/92. The PK crossover comparison study of rFIX and Mononine® indicates that the elimination half-lives are nearly identical (17.7 ± 5.3 hrs for Mononine®), whereas the in vivo recoveries are statistically different (53 ± 12% for Mononine®). The pharmacokinetic parameter estimates for rFIX are consistent across all studies involving previously treated patients. The results of studies of rFIX for on-demand treatment, for prophylaxis, and during surgery in these patients all demonstrate that rFIX has an appropriate efficacy profile indistinguishable from that of high-purity plasma-derived products, indicating that the observed difference in recovery does not require a change in the standard of care for patients when determining an individual's dose during therapy. The amount of BeneFIX™ to be infused, as well as the frequency of infusion, vary with the patient and clinical situation. Estimation of the required dose of BeneFIX™ can be based on the empirical finding that one unit of factor IX activity per kilogram of body weight is expected to increase the circulation level of factor IX by 0.8 IU/dL.

c) Indications

rFIX is a member of the serine protease family of coagulation factors required for hemostasis. The therapeutic indications of rFIX are for control of hemorrhagic episodes and for routine and surgical prophylaxis in patients with hemophilia B (Christmas Disease, i.e. congenital factor IX deficiency). The dosage and duration of the substitution therapy depends on the severity of the disorder of the hemostatic function, on the location and extent of the bleeding disorder, and on the clinical condition. The calculation of the required dosage of rFIX is based on the empirical finding that 1 IU factor IX per kg body weight raises the plasma factor IX activity by approximately 0.8% of normal.

IC:1.2 Expert Report

IC:1.2.1 INTRODUCTION

BeneFIX™ Coagulation Factor IX (Recombinant) [rFIX] is a high purity factor IX preparation comparable to, but not identical with, currently licensed high purity plasma-derived Factor IX [pdFIX] products. On the basis of in vitro clotting assays, the specific activity of rFIX appears to be slightly higher than that of the highest purity pdFIX product (Mononine®). This difference is attributed to the presence of inactive high molecular weight FIX in the Mononine product. rFIX has a primary amino acid sequence that is identical to the Ala^{148} allelic form of pdFIX, and the post-translational modifications of the recombinant molecule appear to be generally similar to those of the plasma-derived molecule. Differences in the extent of gamma-carboxylation (minor), sulfation, phosphorylation, and complexity of carbohydrate structure appear to have little or no effect on in vitro activity or in vivo efficacy (see Preclinical and Clinical Expert Reports). After administration, the recovery of rFIX in patient plasma (0.84 IU/dL per IU/kg) is less than the recovery observed for Mononine (1.17 IU/dL per IU/kg), (see Clinical Expert Report, IC:1.4). This small but reproducible difference in recovery has been attributed to a relatively lower extent of sulfation and lack of phosphorylation of the recombinant FIX (see below and in Preclinical Expert Report, IC:1.3). However, the recovery of rFIX is consistent and within the range empirically observed for factor IX (FIX) products (i.e >0.8 IU/dL per IU/kg; cf. Nov. '92 CPMP *Note for Guidance* (III/3458/92-EN Final) Core summary of product characteristics for human plasma coagulation factor IX concentrate).

Because no human or animal proteins are used in the production or formulation of BeneFIX™, it carries no risk of being contaminated by blood-borne infectious agents, and in contrast to plasma-derived products, its availability is not dependent on donor blood supply. By the nature of the source of BeneFIX™, it is uncontaminated by other plasma-derived clotting factors (e.g.factors II, VII, and X), and consequently rFIX has demonstrated extremely low thrombogenic potential in animal studies (IC:1.3).

IC:1.2.2 MAJOR FINDINGS AND ISSUES

IC:1.2.2.1 COMPOSITION

Like currently marketed FIX products, BeneFIX™ is formulated as a lyophilized dosage form in three strengths: 1000 IU/vial; 500 IU/vial; and 250 IU/vial. Befitting its well-defined drug substance production process, BeneFIX™ contains no human or animal protein in the formulation (Table IIA:1.1-1). Each of the excipients in the rFIX formulation is widely used in the pharmaceutical industry. Because vials are intended for single use, the dosage form contains no preservative. Vials of BeneFIX™ are to be reconstituted in sterile water for injection. The reconstituted solution is appropriately neutral in pH (pH 6.8) and for the two larger dosage strengths is isotonic. Although the reconstituted solution for the 250 IU/vial dose strength is half the osmolality of the larger dosage strengths, there have been no sequelae in the clinical trials (see Clinical Expert Report) or in the stability profile of the reconstituted material (IIF:2f:1.3). The formulation and dosage strengths to be marketed are identical to those used in the clinical trials described in this marketing authorization application (IIA:3).

IC:1.2.2.2 DEVELOPMENT PHARMACEUTICS

Coagulation factors are traditionally administered by intravenous injection. Given the need for hemophiliacs to have constant access to their replacement factor, it is appropriate that rFIX is a lyophilized dosage form which is intended to be stored at 2 to 8 °C, but which nonetheless may be exposed to room temperature for six months without harm (IIF:2g:1). While novel, the formulation is composed of innocuous ingredients widely used in the pharmaceutical industry which provide protection from the stresses of manufacturing and significant stability to both the lyophilized dosage form and the reconstituted material (IIF:2g). In the development of the formulation, appropriate attention has been paid to minimizing the formation of aggregates, which may form under stress conditions (IIC:1.6k) and are specifically observed with other FIX products such as Mononine (see IC:1.2.2.7). The concentrations of the excipients have been optimized during development (IIA:4.1).

IC:1.2.2.3 METHOD OF MANUFACTURE OF DOSAGE FORM

Genetics Institute supplies batches of the frozen (–80 °C) rFIX drug substance to a contract manufacturer (Sanofi Winthrop Pharmaceuticals, McPherson, Kansas, U.S.A.). The duties of the contract manufacturer include control of excipients, formulation, sterile filtration, as well as the aseptic filling and lyophilization of each lot (IIB:2.3a). The three dosage strengths are lyophilized using lyophilization cycles which differ only in their primary drying time (Table IIB:2.4b:5-1). As is true for most proteins, terminal sterilization of BeneFIX™ is impractical because of its sensitivity to sterilizing temperatures (IIA:4.2.4). As appropriate under a contract filling agreement, the filled vials are 100% inspected by the contract manufacturer and samples are taken for sterility and particulate matter testing. Samples are shipped to Genetics Institute for all other release testing. Because the same contract manufacturer was used to manufacture the materials used for clinical trials, for stability studies and for preclinical studies (15 lots; IIB:2.4f:1), the manufacturing relationship between the two companies is well defined.

For distribution in North America, Sanofi Winthrop labels the vials using the clotting activity determined by the Genetics Institute release testing, and ships the finished lots to a distribution center which stores the lots at 2 to 8 °C pending distribution (IIB:2.4b). For distribution in Europe, lots of inkjet-labeled vials are shipped under controlled conditions to Baxter in Belgium, where lots are tested for release, and are then appropriately labeled, packaged and stored at 2 to 8 °C pending distribution. This part of the manufacture of BeneFIX is under the supervision of an appropriately educated Qualified Person.

IC:1.2.2.4 PROCESS VALIDATION

The validation of drug product manufacture is on-going (IIB:3). In order to provide manufacturing flexibility, the validation plan involves examining the robustness of the manufacturing process by manufacturing each of the dosage strengths in two different lyophilizers (IIB:3.2a). In addition, batch sizes of each dosage strength ranging from 2,000 to 10,000 vials are being manufactured (Table IIB:3.2a-2). This matrix approach to some of the variables associated with filling and finishing should provide greater assurance of the robustness of the overall drug product manufacturing process, especially lyophilization, than the alternative approach of repeating the same process multiple times, and is therefore appropriate. Of a total of 9 validation fills planned, 3 have been completed. The data

to date on 15 lots filled during development indicate that the lyophilization process is robust relative to dosage strength, lyophilizer size and batch size (IIB:2.4f:1).

IC:1.2.2.5 CONTROL OF STARTING MATERIALS

Development of FIX.1F Cell Line. The active component in BeneFIX™ Coagulation Factor IX (Recombinant) is the rFIX drug substance. rFIX is synthesized by a genetically engineered CHO cell line. The parent of this production cell line is the well-characterized CHO cell line (IIC:1.3.2c) used to produce many other recombinant glycoproteins, including tPA, EPO, and rAHF, and as such is unremarkable. This host cell was transfected by two separate expression vectors, one containing the full length factor IX cDNA sequence, and the other containing a cDNA sequence encoding a soluble form of a proFIX-processing protease, termed "paired basic amino acid cleavage enzyme" or PACE-SOL (Figure IIC:1.3.2d:1-1). After selection and cloning techniques had been applied, it was found that both expression vectors had stably integrated into the host cell chromosomes, and that each had been amplified to approximately 20 copies per cell (IIC:1.3.3b:4). The integrated, amplified rFIX genes appear to be intact by Southern blot analysis (IIC:1.3.3b:5), Northern blot analysis (IIC:1.3.3b:1) and DNA sequence analysis (IIC:1.3.3b:7).

Although the use of two different expression vectors is unusual, it is not without precedent. The recombinant factor VIII produced by Genetics Institute for Baxter Healthcare Corporation (Recombinate®) also uses a CHO parent cell line engineered with the use of two expression vectors to express recombinant von Willibrand factor (rvWF) and recombinant antihemophilic factor (rAHF).

The primary translation product of the rFIX gene is 461 amino acids in length. Amino terminal cleavage of the secretion peptide within the cell, followed by a further amino terminal cleavage (by endogenous PACE and the expressed PACE-SOL), gives rise to the mature rFIX molecule of 415 amino acids. By cDNA sequence and mass spectrometry of enzymatically generated peptides, the primary amino acid sequence of rFIX has been determined to be identical to that found in Ala^{148} allelic form of pdFIX (IIC:1.6g).

It is remarkable that the production cell line has been adapted to suspension cell culture in growth media that is not only serum-free, but also free of human and animal proteins (Table IIC:1.3.2d:4-1). Other than the proteins secreted by the production cell line, the only protein used in the entire rFIX production process is a recombinant human insulin (either Humulin® or the human insulin analogue Nucellin®) produced in *E.coli*. The adapted cell line has been cryopreserved as a Master Cell Bank (MCB), from which a Working Cell Bank (WCB) has been derived (IIC:1.4). Both the MCB and the WCB have been successfully cryopreserved at –135 °C in the absence of human or animal protein (IIC:1.4a). The MCB, the WCB, and end-of-production (EOP) cells have been characterized (IIC:1.3.3 and IC:1.3.4) according to the appropriate guidelines and found to be stable in genotype and free of any detectable bacterial, mycoplasmal, fungal or viral contamination (IIV:2.3). Although PACE-SOL levels appear to increase 2- to 3-fold over the course of an inoculum run (IIC:1.8a:2.2) due to increasing levels of PACE-SOL mRNA (IIC:1.3.4b:2), this phenotypic change does not appear to be problematic, as there appears to be adequate PACE-SOL at all times to fully process the pro-FIX.

Cell Culture. The production of an active factor IX molecule requires the gamma-carboxylation of glutamic acid residues near the amino terminus of the molecule. Successful gamma-carboxylation in turn requires supplementation of the cell culture medium with appropriate concentrations of Vitamin K_1 (IIC:1.3.2d:7), making the control of this raw material a critical parameter. Vitamin K_1 concentrations in cell culture media are controlled by appropriate handling of the stock solution and careful addition of optimized amounts of Vitamin K_1 to each batch of fresh media in the bioreactor (IIC:1.8a:2.3).

The initial cell culture (~8 population doublings) after thawing several WCB vials uses small quantities of methotrexate (0.1 μM) to maintain selective pressure on rFIX expression. As the cells are passaged through a series of sequentially larger spinner flasks and on into bioreactors, the methotrexate-containing medium is substantially diluted (>100 fold) by medium containing no methotrexate (Tables IIC:1.5.2d:3-1 & IIC:1.5.2d:3-2). The expansion process culminates in one to three 2500-liter stirred tank bioreactors. Production is maintained at this scale using a "batch refeed" process in which approximately 50 to 80% of the bioreactor volume is typically harvested every two to four days and replaced with fresh culture medium (IIC:1.5.2c:2). Conditioned medium from one or more bioreactors is purified to create one batch of rFIX drug substance. This culture process has been run continuously for up to 58 cell population doublings from the WCB (~17 batches of drug substance), over a period of approximately two months from a single inoculum (IIC:1.5.2g). Cell generations are tracked for each bioreactor, and based on maximal cumulative population doublings used to date, Genetics Institute has appropriately imposed within each inoculum run a process limit (Table IIC:1.5.2f:1.2-1) of 58 generations from the WCB for cells from any bioreactor harvested to make a batch of drug substance. Material made throughout the course of an inoculum run is comparable by all analytical tests, except possibly for a slight trend (up to 10%) towards increasing specific activity (IIC:1.10).

Other in-process cell culture monitoring for each bioreactor harvested includes cell viability, cell density, cell doubling time, cellular productivity for rFIX, pH, temperature, dissolved oxygen, bioburden and mycoplasma. In addition, for each inoculum run there is post-production testing (on end-of-production cells, not on each bioreactor harvest) for bacterial, mycoplasmal, fungal and viral contamination (Table IIC:1.5.2f:1.2-1). This in-process monitoring is conventional and in compliance with the expectations of regulatory guidelines (ICH Draft Guideline on Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin).

Purification. The purification of rFIX does not involve the use of a monoclonal antibody column and its attendant complications, such as the potential for contamination by antibody and murine viruses, or the need for denaturing elution conditions. Instead, the purification uses a series of four chromatography resins (Figure IIC:1.5.3c-1) and takes advantage of intrinsic properties of the rFIX molecule to adsorb and desorb the molecule under mild conditions. Each of the resins is standard to the pharmaceutical industry. The first chromatographic step is designed as a pseudo-affinity chromatography step to be highly selective. rFIX in diafiltered and concentrated conditioned medium is loaded on a quaternary amine resin (Q Sepharose Fast Flow) and the rFIX is subsequently eluted with a low concentration (10 mM) of calcium chloride (IIC:1.5.3c:1). The calcium ions induce a conformational change in rFIX that results in selective elution from the resin. Subsequent to the Q Sepharose step, there are three additional chromatography steps.

In an effort to provide extraordinary assurance to end users and their physicians who are worried about unknown and currently undetectable infectious agents, a virus-retaining filtration step (Viresolve™, molecular weight cutoff 70,000) which allows passage of rFIX has been incorporated into the process since the middle of the clinical trials. This has been accomplished with minimal loss of rFIX (Table IIC:1.5.3i:2-1) and no adverse effects on the resulting drug substance or drug product.

Process Validation. Consistent with ICH Guidelines, the rFIX production process is characterized by a multifaceted approach to viral safety, including (1) the control of raw materials, (2) analyzed purity of the cell banks, (3) rigorous post-process virus testing, (4) control of the manufacturing conditions, and (5) validation of the virus-removing capacity of the other purification steps (IIV). The virus-retaining filtration step provides approximately 10^5 -fold removal of appropriately diverse model viruses (amphotropic murine leukemia virus, bovine parvovirus, human herpes simplex virus type I, parainfluenza virus, reovirus type 3) (Table IIC:1.8b:2-1). In addition to the virus-retaining filtration step, two other purification steps (Q Sepharose FF and Chelate-EMD-Cu^{++}) have been investigated for their ability to remove model viruses. The overall fold-removal from these three steps (a minimum of 10^{10}-fold for each model virus) is sufficient to provide assurance of removal of any adventitious viral contamination not otherwise detectable during post-process virus testing (Section IIV:4.1).

Other process validation studies include removal of host cell proteins (~10^4-fold; IIC:1.8b:3.2), PACE (~10^5-fold; IIC:1.8b:3.3), rhInsulin (>100-fold through the Q-Sepharose step alone; IIC:1.8b:4.6), methotrexate (>100-fold by the Q Sepharose step alone; IIC:1.8b:4.1) and DNA (>10^9-fold; IIC:1.8b:3.1) as well as many other process components (IIC:1.8). These studies have indicated that each of these potential contaminants is reproducibly removed to low, nonproblematic levels. The purification process has not been validated for the removal of proFIX; however, given the consistently low levels of proFIX in cell culture media (Figure IIC:1.8a:2.2-7), the validation of proFIX processing (IIC:1.8a:2.2), and the consistently low levels of proFIX measured in the drug substance (Figure IIC:1.8a:2.2-5), this omission is not problematic. As mentioned below, the removal of copper ions during the final ultrafiltration/diafiltration has been validated (IIC:1.8b:5.3).

Data from in-process monitoring indicate that the purification process has reproducible yields at each step (IIC:1.5.3i:2). In-process control of the purification process includes pH measurement of all buffers (IIC:1.5.4d). In addition to substantial manufacturing experience (4 inocula; 48 batches) at the one-bioreactor scale, 17 batches have been manufactured from one inoculum at a larger (2 and 3 bioreactor) scale. All in-process monitoring and drug substance release testing results have indicated that the manufacturing process is robust and reproducible (IIC:1.5.2g; IIC:1.5.3i; IIC:1.10).

Although the virus-retaining filters are single-use, the column chromatography resins and the microfiltration and ultrafiltration/diafiltration membranes are reused after cleaning with appropriate cleaning agents (i.e. NaOH or NaOCl). Having thus far seen no deterioration in resin or membrane performance (IIC:1.8d and IIC:1.8e), Genetics Institute has as yet placed no limitation on the number of times the resins and membranes may be reused. This is acceptable because there is an on-going program in place to assess the suitability of resin and membrane reuse and ultimate life.

Drug Substance Specifications and Release Testing. Drug substance release testing (Table IIC:1.1a:1-1) includes appropriately specific and sensitive tests for: purity (SDS-PAGE, RP-HPLC, SEC); safety (endotoxin, bioburden); potency (clotting assay); concentration (SEC); specific activity (activity/concentration); and identity (extent of gamma-carboxylation, peptide mapping, N-glycan carbohydrate fingerprinting, and SDS-PAGE). IEF analysis for carbohydrate charge distribution was not necessary due to the superior resolving power and structural information obtained from N-glycan carbohydrate fingerprinting.

Using standard electrophoretic and HPLC-based assays, specifications have been set which restrict the amount of the total detectable non-rFIX protein species to ≤1%. Results of testing to date have indicated very low levels (≤0.6%) of non-rFIX impurities (IIC:1.9b:6). The only potential non-rFIX protein contaminants that are possible for this manufacturing process are rhInsulin, PACE and host cell proteins. As mentioned above, validation studies have indicated that these proteins are consistently removed. Preclinical and clinical data have indicated that BeneFIX™ is well tolerated and have not indicated a need for further release specifications (see Preclinical and Clinical expert reports).

Genetics Institute has chosen to validate the removal of DNA and host cell proteins (IIC:1.8b:3), rather than implement specific routine release assays. The DNA and host cell protein validations examined the historical norms for the amounts of these materials in conditioned media from rFIX manufacture and found them to be consistent. In addition, drug substance purity measurements (SDS-PAGE and HPLC) have been examined for their ability to detect host cell proteins at levels which would indicate failure of the manufacturing process. This approach is not typical of industry practice, but is appropriate given the growing consensus that neither DNA nor host cell proteins represent a safety risk (FDA Workshop on Characterization of Biotechnology Pharmaceutical Products, Washington, D.C. December, 1995).

Similarly, Genetics Institute has chosen to validate that PACE will be removed during the purification process (IIC:1.8b:3.3) rather than employ a routine release assay. Given the extent and reproducibility of the manufacturing process data to date, the implementation of specific drug substance release assays for PACE is not needed.

The final chromatography column contains copper ions. Genetics Institute has performed concurrent validation studies which demonstrate that the diafiltration step will remove excess copper ions >100-fold. No drug substance assay or specification for copper is proposed. To date, copper ions have been detected (limit of detection 0.01 μg/mL) in 33 of 65 drug substance batches, and 11 of these 33 contained quantifiable levels (> 0.05 μg/mL) of up to 0.29 μg/mL (Table IIC:1.9b:6-1). The copper levels in these batches have not been observed to be problematic to the specific activity (Table IIC:1.10b-3) of the drug substance.

Preclinical and clinical investigations have indicated that the in vivo recovery of BeneFIX™ is consistently lower than Mononine™. Genetics Institute has been able to attribute this lower recovery to a lack of phosphorylation and a relative lack of sulfation of the recombinant molecule, and have established lot-to-lot consistency of recovery of rFIX in both an animal model and in human clinical trials (see Preclinical and Clinical Expert Reports). Because the maximum possible effect on recovery

is slight and because the lot-to-lot consistency of BeneFIX™ has been established, Genetics Institute is justified in not establishing release assays for sulfation, phosphorylation, or in vivo recovery.

Characterization of the rFIX Molecule. Extensive characterization of the rFIX drug substance (IIC:1.6) has indicated that although it is comparable to pdFIX, the rFIX molecule is not identical to the pdFIX molecule. As described above, the primary amino acid sequences of the two molecules are identical, although there is allelic heterogeneity in the pdFIX molecule at the Ala/Thr^{148} site. Both molecules are β-hydroxylated (~48% for rFIX; ~37% for pdFIX) at Asp^{64} (in the first EGF domain) and both are sulfated at Tyr^{155} (in the activation peptide), although pdFIX is sulfated to a greater extent (>90%) at this position than rFIX (~25%) (Figure IIC:1.6h:3.2-2).

The primary differences between the rFIX and pdFIX are that: the rFIX molecule is not phosphorylated at Ser^{158} (in the activation peptide), while the pdFIX is substantially phosphorylated; the pdFIX molecule appears to have more heterogeneity in N-linked carbohydrate structures than the rFIX molecule; pdFIX is fully gamma-carboxylated at all 12 sites, whereas rFIX is somewhat under-gamma-carboxylated at two of the twelve sites (mostly at Glu^{40} and to a lesser extent at Glu$^{36)}$. None of these differences appears to affect the in vitro clotting activity of the rFIX molecule (IIC:1.6h), nor is there an effect on efficacy or on the half life in patient plasma (see Clinical expert report). The recoveries of pdFIX and rFIX in patient plasma are both lower than the recoveries observed for factor VIII (~100%), with the recovery of rFIX (~38%) being less than that observed for Mononine (~51%) (see Clinical Expert Report). As a result of extensive investigations of these phenomena, these differences in recovery have been attributed primarily to the differences in sulfation and phosphorylation of the two molecules (see Preclinical Expert Report).

Higher order structure investigations in the presence and absence of calcium ions using circular dichroism (both near and far UV), tryptophan fluorescence, and sedimentation velocity ultracentrifugation have indicated that rFIX is indistinguishable from pdFIX from which high molecular weight species have been removed (IIC:1.6i).

The excipients used to formulate the finished product meet national and international compendial requirements and therefore have appropriate specifications and test methods (IIC:2.1.1).

IC:1.2.2.6 CONTROL TESTS ON INTERMEDIATE PRODUCTS

Not applicable.

IC:1.2.2.7 CONTROL TESTS ON THE FINISHED PRODUCT

Each lot of BeneFIX™ is filled on the basis of potency as measured in an in-process single-stage clotting assay (IIC:2.4b:2). Samples of each filled and lyophilized lot are then reconstituted and tested for potency using the single-stage clotting assay, and the lot is labeled on the basis of this potency assay. The chromogenic assay described in the European Pharmacopoeia is not used. After reconstitution, the results of the clotting assay and the determination of protein concentration (by SEC) allow a sufficiently accurate determination of the specific activity of each lot of rFIX to reasonably control the product potency.

Because lyophilization has the potential to form aggregates in protein preparations, each lot of BeneFIX™ is assayed for high molecular weight (HMW) material by an SEC assay. Although Genetics Institute has shown that lots of pdFIX have exhibited amounts of inactive aggregates in the range of 10 to 20% of total protein (IIC:1.6i:1), the manufacturing experience to date with BeneFIX™ (15 lots) has been reproducibly ≤1% (Table IIE:2.2c-2). Because HMW material has been observed at up to 1.4% for the drug substance, and given the potential for the lyophilization process and the storage of the lyophilized product to lead to aggregate formation, the high molecular weight species specification has been set at ≤4% for the drug product, versus ≤3% for the drug substance. As a consequence of the relative absence of HMW material in BeneFIX™ compared with Mononine™, the observed specific activity of the rFIX is correspondingly higher, ~260 IU/mg for BeneFIX™ versus ~220 IU/mg for Mononine™ (Table IIC:1.6i:1-1). As a further consequence, the specification for the specific activity of BeneFIX™ (200 to 360 IU/mg) has been established to be slightly higher than that for Mononine™, ≥150 IU/mg (U.S. FDA Summary of Basis for Approval).

To guard against degradation or contamination of rFIX during the filling process, the SDS-PAGE purity assay is used for each drug product lot, using the same specification for impurities (≤1%; Table IIE:1.1.1-1) as for the rFIX drug substance.

Additional specifications for appearance (before and after reconstitution), pH, residual moisture, particulate matter, activated FIX, bacterial endotoxin and sterility meet compendial expectations (Table IIE:1.1.1-1). No specification for the General Safety Test (in the U.S.) or Abnormal Toxicity (in the E.C.) is set, as is appropriate for such a well controlled manufacturing process and such a well characterized molecule. Having tested the clinical lots of rFIX in both the Limulus Amoebocyte Lysate (LAL) assay for endotoxin as well as the pyrogen test in rabbits, the decision to test the marketed product solely by the validated LAL test is appropriately sparing of test animals.

There is no release test of the finished product such as isoelectric focussing that directly indicates the charge of the rFIX molecule. Monitoring of this parameter is indirectly accomplished at the drug substance stage using carbohydrate fingerprinting of N-linked carbohydrate (extent of sialylation) and ion-exchange resin elution profiles (Gla content). Stability studies (see below) have indicated that neither the lyophilization process nor storage of the drug substance or drug product under the recommended conditions appears to affect this parameter.

In general, the release tests and specifications for BeneFIX™ are consistent with the analytical validation results (see below), lot release data to date (Table IIE:2.2c-2), stability studies (see below), and regulatory guidelines (IIE:1.1.2).

Genetics Institute proposes to package individual vials of BeneFIX™ in a kit containing a vial of commercially available Sterile Water for Injection (for reconstitution of the BeneFIX™) and appropriate sterile withdrawal and infusion devices (IA:2.2).

IC:1.2.2.8 ANALYTICAL VALIDATION

As recommended in the ICH Guideline on Validation of Analytic Procedures, all release assays have been appropriately validated to be precise, reproducible, and sensitive, given the product specifications (IIE:2.1). The clotting activity assay has been developed using working reference

material composed of pooled normal plasma and calibrated against the NIBSC International Standard for Blood Coagulation Factor IX, Concentrate (IIE:1.2.1d). Other assays use an appropriately qualified rFIX reference material (IIC:1.7c:2). The effects of sample matrix on each assay have been investigated and are understood. These validation studies (IIE:2.1) indicate that the release assays are robust.

IC:1.2.2.9 STABILITY

Forced decomposition studies (IIC:1.6k) have been used to determine the potential degradation pathways for the rFIX molecule. These studies have shown that degradation products would have been detected in the real-time stability studies, had they occurred.

Using rFIX from the one-bioreactor process, the stability of the drug substance (3 lots at –80 °C) and the drug product (3 lots of 1000 IU/vial; 1 lot of 500 IU/vial; 3 lots of 250 IU/vial; all at 2 to 8 °C) have been investigated. Given that the formulation of the three dosage strengths is identical, the bracketing approach used for stability studies is consistent with the ICH guideline on stability testing.

Data to date (IIF) indicate that the drug substance is stable for at least 18 months at –80 °C (IIF:1e:1) and the drug product is stable for at least 18 months at 2 to 8 °C (IIF:2g:1). The lyophilized product is also stable for at least 6 months at room temperature (IIF:2f). Studies of the reconstituted material have indicated that it is stable for at least 24 hours at room temperature (IIF:2g:2).

Given the linear nature of the scale-up of the process and the biochemical comparability of the multiple bioreactor process material to the one bioreactor process material, setting the expiration dating for the drug substance at 18 months and for the drug product at 18 months based on the data from the one bioreactor process is appropriate and in conformance with ICH guidelines. Extension of this expiration dating will be appropriate when later data become available. Genetics Institute has committed to and has begun confirmatory studies that will make use of lots of drug substance and lots of drug product manufactured using the multiple bioreactor process (IIF:1g and IIF:2h). These confirmatory stability data are to be achieved by putting one lot of drug substance per year and one lot of drug product per year on the confirmatory testing program (IIF:2h).

The effects of freeze-thaw, light, shipping conditions and elevated temperature (40 °C) on BeneFIX™ have all been appropriately investigated. Studies have indicated (IIF:2c:2.5) that the only instability observed for the lyophilized rFIX product appears to be a slight tendency to form high molecular weight material (aggregates) at elevated temperature (<1% over 3 months at 40 °C). There is no evidence that aggregates form under the recommended storage conditions.

IC:1.2.2.10 EVALUATION CONCLUSIONS

Factor IX is a very complex, highly posttranslationally modified molecule. Although very similar, the rFIX produced by Genetics Institute is not identical to the corresponding pdFIX. Nonetheless, rFIX is potent, pure, and stable, and as such is appropriate for its intended use. Both animal data and human clinical trial data (see corresponding expert reports) further support this conclusion.

The manufacturing process for the active ingredient of rFIX is well defined, under good control, robust, and follows current good manufacturing practices for recombinant DNA-based products. Because the cell banks have been well characterized and all other raw materials are essentially chemically defined, there is extremely little chance of contamination of rFIX by infectious agents. By contrast, the manufacture of the plasma-derived material is dependant on the blood supply, with each lot stemming from tens of thousands of different donors, each of whom has the potential for introducing both detectable and undetectable blood-borne pathogens into the production system. By design, rFIX is thus significantly safer than pdFIX with regard to blood-borne pathogens. For the same reasons, it is also not surprising that rFIX is more homogeneous than pdFIX.

The tests chosen to control the manufacture of the active component and the final dosage form address the issues of purity, potency, identity, and safety. These tests have been appropriately validated and are appropriately controlled by the use of reference standards. The most critical test is the traditional clotting activity assay, in which the rFIX molecule must interact correctly with all components of the tenase complex (calcium ions, phospholipid, factor VIIIa, and factor X) to produce factor Xa. This activity assay is extremely relevant to the clinical use of the product, and the specific activity of the rFIX molecule in this assay is an excellent indicator that the rFIX molecule has the appropriate attributes for use in treating hemophilia B.

Using release testing, rFIX from the multiple bioreactor process is indistinguishable from rFIX from the earlier 1 bioreactor process that was used for most of the clinical trials (IIC:1.10b:1). Although rFIX demonstrates somewhat lower recoveries in patient plasma than Mononine, these recoveries are reproducible, consistent lot-to-lot and appropriate for the treatment of bleeding episodes (see Clinical and Preclinical Expert Reports).

The rFIX dosage form is appropriately stable for its intended use. The dosage form and strengths chosen are traditional to the hemophilia B population.

IC:1.2.3 REFERENCES

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IC:1.2.4 INFORMATION ON THE EXPERT

Frederick T. Gates III

Frederick T. Gates, III Ph.D.

July 26th, 1996
Date

Dr. Gates received a B.S. degree in Molecular Biophysics and Biochemistry from Yale University in 1971, and a Ph.D. in Biochemistry from the University of California at Berkeley, in 1976. After postdoctoral studies at Rockefeller University and the National Institute for Allergy and Infectious Diseases of the NIH, in 1980 Dr. Gates joined what was then the Office of Biologics of the Food and Drug Administration as a Senior Staff Fellow. While at the FDA, Dr. Gates conducted research in the molecular biology of the immune system, coauthored a number of Points to Consider documents, reviewed Investigational New Drug and Product Licencing Applications, and inspected manufacturers of recombinant biological products.

In 1987 Dr. Gates (by then a Research Chemist with the Center for Biologics Evaluation and Research (CBER)) left the FDA and joined Genetics Institute as Head of Regulatory Affairs. From 1990 to 1994, he served as the industry representative to the FDA's Blood Products Advisory Committee. Currently, Dr. Gates is Director of Regulatory Affairs at Genetics Institute, and he has been instrumental in developing the Chemistry, Manufacturing and Control information for a number of Genetics Institute's products, including rAHF (Recombinate®), EPO (Recormon®/Epogin®), and rFIX (BeneFIX™).

A curriculum vitae for Dr. Gates follows.

July, 1996

CURRICULUM VITAE

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Address:	7 Trotting Road, Chelmsford, MA 01824	
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Marital Status:	Married, two sons	

Education:

1971	B.S., Molecular Biophysics and Biochemistry, Yale University
1976	Ph.D., Biochemistry, University of California, Berkeley

Appointments:

1992-present	Director, Regulatory Affairs, Genetics Institute, Inc. 87 CambridgePark Drive, Cambridge, MA 02140
1990-1994	Industry Representative, Blood Products Advisory Committee, U.S. Food and Drug Administration
1989-1992	Associate Director, Regulatory Affairs, Genetics Institute, Inc. 87 CambridgePark Drive, Cambridge, MA 02140
1987-1989	Head, Regulatory Affairs, Genetics Institute, Inc. 87 CambridgePark Drive, Cambridge, MA 02140
1983-1987	Research Chemist, Food and Drug Administration, Center for Drugs and Biologics, Office of Biologics Research and Review, Division of Biochemistry and Biophysics, Cell Biology Branch, Bethesda, MD 20892
1980-1983	Senior Staff Fellow, Food and Drug Administration, Bureau of Biologics, Division of Biochemistry and Biophysics, Bethesda, MD 20892

1977-1980	Staff Fellow, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Immunogenetics, Bethesda, MD 20892
1976-1977	Postdoctoral Fellow (NIH Fellowship) The Rockefeller University, New York
1971-1976	Ph.D. Candidate in Department of Biochemistry, University of California, Berkeley
1970	NSF Undergraduate Research Program, Yale University

Society Memberships:
Harvey Society
American Association of Immunologists
Regulatory Affairs Professional Society

Honors:

1966-1967	Dalton Prize in Chemistry
1971-1976	Predoctoral Trainee, NIH
1976-1977	Postdoctoral Fellow, NIH
1987	Commendable Service Award, FDA

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Part IC EXPERT REPORTS

IC:1 Chemical, Pharmaceutical, and Biological Expert Report

IC:1.1 Product Profile

a) Type of application: a new active substance

b) Chemical and pharmacokinetic properties

Chemical

BeneFIX™, Coagulation Factor IX (Recombinant) [rFIX; also referred to as rhFIX] is a 415-amino acid glycoprotein (approximately 55 kDa) that is produced in Chinese hamster ovary (CHO) cells (FIX.1F cell line) and subsequently very highly purified using a four-step chromatography process. For maximum assurance of safety, the purification process also contains a viral filtration step capable of removing viruses. Other than use of the FIX.1F cell line and recombinant human insulin made in *E.coli*, no animal or human proteins are used in the manufacture and purification of rFIX. The rFIX drug product is a lyophilized powder for injection cake produced from a solution containing approximately 100 IU rFIX/mL, 10 mM L-histidine, 0.26 M glycine, 1% sucrose, and 0.005% polysorbate 80, pH 6.8. The lyophilized formulation is stable, contains no preservatives, and contains no added animal or human raw materials. The drug product is available in 1000-, 500-, and 250-IU dosage strengths which are approximately 100-, 100-, and 50-IU rFIX/mL, respectively, when reconstituted with Sterile Water for Injection as directed.

The amino acid sequence of rFIX is identical to that of the Ala 148 allelic form of plasma-derived FIX (pdFIX). Total impurities account for ≤1%, as determined by SDS-PAGE. The specific activity of rFIX, as determined by the traditional one-step clotting assay, is approximately 260 IU/mg.

With respect to post-translational modifications, rFIX contains approximately 11.5 γ-carboxyglutamyl (Gla) residues, with full occupancy of the Gla residues important for calcium binding. rFIX is glycosylated via both N- and O-linkages. The N-linked oligosaccharides of rFIX are of the complex type, with structures ranging from tetrasialylated to neutral. Partial β-hydroxylation of Asp^{64} is inferred from peptide mapping and mass spectrometric analysis. Partial sulfation of rFIX occurs at Tyr^{155}. The amino acid residues of rFIX are not oxidized or phosphorylated, whereas a low degree of deamidation may be present.

Pharmacokinetic Parameters and Clinical Efficacy

Animal. Pharmacology studies of rFIX in a canine model of hemophilia B indicate that rFIX can correct genetically deficient hemostasis. Evaluation of rFIX in the modified Wessler Stasis model in rabbits has shown rFIX to have very low thrombogenicity relative to commercially available forms of human plasma-derived FIX (pdFIX). Pharmacokinetic parameters of rFIX have been determined after single and multiple intravenous doses in Sprague-Dawley rats, beagle dogs, and mixed-breed

hemophilia B dogs. The PK parameter estimates were linear across all doses evaluated (40 to 200 IU/kg), and accumulation of rFIX was not found. The PK parameters obtained in the studies in dogs and rats that compared rFIX to pdFIX (Mononine®, Armour Pharmaceuticals, Inc.) are similar to those obtained in human studies (see below). In studies investigating how structural features of rFIX and pdFIX affect recovery, it was determined that charged moieties on the activation peptide contribute to the modulation of recovery within the range observed for rFIX and pdFIX.

Human. In a crossover pharmacokinetic evaluation of rFIX and Mononine® in previously treated patients (n=11), infusion of rFIX into patients with hemophilia B resulted inin vivo recoveries of approximately 38 ± 14%. The plasma half-life of factor IX was 18.1 ± 5.1 hours. The observed 0.84 IU/dL rise in rFIX activity per IU/kg of rFIX administered is within the range outlined in the core Summary of Product Characteristics (SmPC) for human coagulation IX concentrates in the European Community (EC) *Note for Guidance* III/3458/92. The PK crossover comparison study of rFIX and Mononine® indicates that the elimination half-lives are nearly identical (17.7 ± 5.3 hrs for Mononine®), whereas the in vivo recoveries are statistically different (53 ± 12% for Mononine®). The pharmacokinetic parameter estimates for rFIX are consistent across all studies involving previously treated patients. The results of studies of rFIX for on-demand treatment, for prophylaxis, and during surgery in these patients all demonstrate that rFIX has an appropriate efficacy profile indistinguishable from that of high-purity plasma-derived products, indicating that the observed difference in recovery does not require a change in the standard of care for patients when determining an individual's dose during therapy. The amount of BeneFIX™ to be infused, as well as the frequency of infusion, vary with the patient and clinical situation. Estimation of the required dose of BeneFIX™ can be based on the empirical finding that one unit of factor IX activity per kilogram of body weight is expected to increase the circulation level of factor IX by 0.8 IU/dL.

c) Indications

rFIX is a member of the serine protease family of coagulation factors required for hemostasis. The therapeutic indications of rFIX are for control of hemorrhagic episodes and for routine and surgical prophylaxis in patients with hemophilia B (Christmas Disease, i.e. congenital factor IX deficiency). The dosage and duration of the substitution therapy depends on the severity of the disorder of the hemostatic function, on the location and extent of the bleeding disorder, and on the clinical condition. The calculation of the required dosage of rFIX is based on the empirical finding that 1 IU factor IX per kg body weight raises the plasma factor IX activity by approximately 0.8% of normal.

IC:1.2 Expert Report

IC:1.2.1 INTRODUCTION

BeneFIX™ Coagulation Factor IX (Recombinant) [rFIX] is a high purity factor IX preparation comparable to, but not identical with, currently licensed high purity plasma-derived Factor IX [pdFIX] products. On the basis of in vitro clotting assays, the specific activity of rFIX appears to be slightly higher than that of the highest purity pdFIX product (Mononine®). This difference is attributed to the presence of inactive high molecular weight FIX in the Mononine product. rFIX has a primary amino acid sequence that is identical to the Ala^{148} allelic form of pdFIX, and the post-translational modifications of the recombinant molecule appear to be generally similar to those of the plasma-derived molecule. Differences in the extent of gamma-carboxylation (minor), sulfation, phosphorylation, and complexity of carbohydrate structure appear to have little or no effect on in vitro activity or in vivo efficacy (see Preclinical and Clinical Expert Reports). After administration, the recovery of rFIX in patient plasma (0.84 IU/dL per IU/kg) is less than the recovery observed for Mononine (1.17 IU/dL per IU/kg), (see Clinical Expert Report, IC:1.4). This small but reproducible difference in recovery has been attributed to a relatively lower extent of sulfation and lack of phosphorylation of the recombinant FIX (see below and in Preclinical Expert Report, IC:1.3). However, the recovery of rFIX is consistent and within the range empirically observed for factor IX (FIX) products (i.e >0.8 IU/dL per IU/kg; cf. Nov. '92 CPMP *Note for Guidance* (III/3458/92-EN Final) Core summary of product characteristics for human plasma coagulation factor IX concentrate).

Because no human or animal proteins are used in the production or formulation of BeneFIX™, it carries no risk of being contaminated by blood-borne infectious agents, and in contrast to plasma-derived products, its availability is not dependent on donor blood supply. By the nature of the source of BeneFIX™, it is uncontaminated by other plasma-derived clotting factors (e.g.factors II, VII, and X), and consequently rFIX has demonstrated extremely low thrombogenic potential in animal studies (IC:1.3).

IC:1.2.2 MAJOR FINDINGS AND ISSUES

IC:1.2.2.1 COMPOSITION

Like currently marketed FIX products, BeneFIX™ is formulated as a lyophilized dosage form in three strengths: 1000 IU/vial; 500 IU/vial; and 250 IU/vial. Befitting its well-defined drug substance production process, BeneFIX™ contains no human or animal protein in the formulation (Table IIA:1.1-1). Each of the excipients in the rFIX formulation is widely used in the pharmaceutical industry. Because vials are intended for single use, the dosage form contains no preservative. Vials of BeneFIX™ are to be reconstituted in sterile water for injection. The reconstituted solution is appropriately neutral in pH (pH 6.8) and for the two larger dosage strengths is isotonic. Although the reconstituted solution for the 250 IU/vial dose strength is half the osmolality of the larger dosage strengths, there have been no sequelae in the clinical trials (see Clinical Expert Report) or in the stability profile of the reconstituted material (IIF:2f:1.3). The formulation and dosage strengths to be marketed are identical to those used in the clinical trials described in this marketing authorization application (IIA:3).

IC:1.2.2.2 DEVELOPMENT PHARMACEUTICS

Coagulation factors are traditionally administered by intravenous injection. Given the need for hemophiliacs to have constant access to their replacement factor, it is appropriate that rFIX is a lyophilized dosage form which is intended to be stored at 2 to 8 °C, but which nonetheless may be exposed to room temperature for six months without harm (IIF:2g:1). While novel, the formulation is composed of innocuous ingredients widely used in the pharmaceutical industry which provide protection from the stresses of manufacturing and significant stability to both the lyophilized dosage form and the reconstituted material (IIF:2g). In the development of the formulation, appropriate attention has been paid to minimizing the formation of aggregates, which may form under stress conditions (IIC:1.6k) and are specifically observed with other FIX products such as Mononine (see IC:1.2.2.7). The concentrations of the excipients have been optimized during development (IIA:4.1).

IC:1.2.2.3 METHOD OF MANUFACTURE OF DOSAGE FORM

Genetics Institute supplies batches of the frozen (–80 °C) rFIX drug substance to a contract manufacturer (Sanofi Winthrop Pharmaceuticals, McPherson, Kansas, U.S.A.). The duties of the contract manufacturer include control of excipients, formulation, sterile filtration, as well as the aseptic filling and lyophilization of each lot (IIB:2.3a). The three dosage strengths are lyophilized using lyophilization cycles which differ only in their primary drying time (Table IIB:2.4b:5-1). As is true for most proteins, terminal sterilization of BeneFIX™ is impractical because of its sensitivity to sterilizing temperatures (IIA:4.2.4). As appropriate under a contract filling agreement, the filled vials are 100% inspected by the contract manufacturer and samples are taken for sterility and particulate matter testing. Samples are shipped to Genetics Institute for all other release testing. Because the same contract manufacturer was used to manufacture the materials used for clinical trials, for stability studies and for preclinical studies (15 lots; IIB:2.4f:1), the manufacturing relationship between the two companies is well defined.

For distribution in North America, Sanofi Winthrop labels the vials using the clotting activity determined by the Genetics Institute release testing, and ships the finished lots to a distribution center which stores the lots at 2 to 8 °C pending distribution (IIB:2.4b). For distribution in Europe, lots of inkjet-labeled vials are shipped under controlled conditions to Baxter in Belgium, where lots are tested for release, and are then appropriately labeled, packaged and stored at 2 to 8 °C pending distribution. This part of the manufacture of BeneFIX is under the supervision of an appropriately educated Qualified Person.

IC:1.2.2.4 PROCESS VALIDATION

The validation of drug product manufacture is on-going (IIB:3). In order to provide manufacturing flexibility, the validation plan involves examining the robustness of the manufacturing process by manufacturing each of the dosage strengths in two different lyophilizers (IIB:3.2a). In addition, batch sizes of each dosage strength ranging from 2,000 to 10,000 vials are being manufactured (Table IIB:3.2a-2). This matrix approach to some of the variables associated with filling and finishing should provide greater assurance of the robustness of the overall drug product manufacturing process, especially lyophilization, than the alternative approach of repeating the same process multiple times, and is therefore appropriate. Of a total of 9 validation fills planned, 3 have been completed. The data

to date on 15 lots filled during development indicate that the lyophilization process is robust relative to dosage strength, lyophilizer size and batch size (IIB:2.4f:1).

IC:1.2.2.5 CONTROL OF STARTING MATERIALS

Development of FIX.1F Cell Line. The active component in BeneFIX™ Coagulation Factor IX (Recombinant) is the rFIX drug substance. rFIX is synthesized by a genetically engineered CHO cell line. The parent of this production cell line is the well-characterized CHO cell line (IIC:1.3.2c) used to produce many other recombinant glycoproteins, including tPA, EPO, and rAHF, and as such is unremarkable. This host cell was transfected by two separate expression vectors, one containing the full length factor IX cDNA sequence, and the other containing a cDNA sequence encoding a soluble form of a proFIX-processing protease, termed "paired basic amino acid cleavage enzyme" or PACE-SOL (Figure IIC:1.3.2d:1-1). After selection and cloning techniques had been applied, it was found that both expression vectors had stably integrated into the host cell chromosomes, and that each had been amplified to approximately 20 copies per cell (IIC:1.3.3b:4). The integrated, amplified rFIX genes appear to be intact by Southern blot analysis (IIC:1.3.3b:5), Northern blot analysis (IIC:1.3.3b:1) and DNA sequence analysis (IIC:1.3.3b:7).

Although the use of two different expression vectors is unusual, it is not without precedent. The recombinant factor VIII produced by Genetics Institute for Baxter Healthcare Corporation (Recombinate®) also uses a CHO parent cell line engineered with the use of two expression vectors to express recombinant von Willibrand factor (rvWF) and recombinant antihemophilic factor (rAHF).

The primary translation product of the rFIX gene is 461 amino acids in length. Amino terminal cleavage of the secretion peptide within the cell, followed by a further amino terminal cleavage (by endogenous PACE and the expressed PACE-SOL), gives rise to the mature rFIX molecule of 415 amino acids. By cDNA sequence and mass spectrometry of enzymatically generated peptides, the primary amino acid sequence of rFIX has been determined to be identical to that found in Ala^{148} allelic form of pdFIX (IIC:1.6g).

It is remarkable that the production cell line has been adapted to suspension cell culture in growth media that is not only serum-free, but also free of human and animal proteins (Table IIC:1.3.2d:4-1). Other than the proteins secreted by the production cell line, the only protein used in the entire rFIX production process is a recombinant human insulin (either Humulin® or the human insulin analogue Nucellin®) produced in *E.coli*. The adapted cell line has been cryopreserved as a Master Cell Bank (MCB), from which a Working Cell Bank (WCB) has been derived (IIC:1.4). Both the MCB and the WCB have been successfully cryopreserved at –135 °C in the absence of human or animal protein (IIC:1.4a). The MCB, the WCB, and end-of-production (EOP) cells have been characterized (IIC:1.3.3 and IC:1.3.4) according to the appropriate guidelines and found to be stable in genotype and free of any detectable bacterial, mycoplasmal, fungal or viral contamination (IIV:2.3). Although PACE-SOL levels appear to increase 2- to 3-fold over the course of an inoculum run (IIC:1.8a:2.2) due to increasing levels of PACE-SOL mRNA (IIC:1.3.4b:2), this phenotypic change does not appear to be problematic, as there appears to be adequate PACE-SOL at all times to fully process the pro-FIX.

Cell Culture. The production of an active factor IX molecule requires the gamma-carboxylation of glutamic acid residues near the amino terminus of the molecule. Successful gamma-carboxylation in turn requires supplementation of the cell culture medium with appropriate concentrations of Vitamin K_1 (IIC:1.3.2d:7), making the control of this raw material a critical parameter. Vitamin K_1 concentrations in cell culture media are controlled by appropriate handling of the stock solution and careful addition of optimized amounts of Vitamin K_1 to each batch of fresh media in the bioreactor (IIC:1.8a:2.3).

The initial cell culture (~8 population doublings) after thawing several WCB vials uses small quantities of methotrexate (0.1 μM) to maintain selective pressure on rFIX expression. As the cells are passaged through a series of sequentially larger spinner flasks and on into bioreactors, the methotrexate-containing medium is substantially diluted (>100 fold) by medium containing no methotrexate (Tables IIC:1.5.2d:3-1 & IIC:1.5.2d:3-2). The expansion process culminates in one to three 2500-liter stirred tank bioreactors. Production is maintained at this scale using a "batch refeed" process in which approximately 50 to 80% of the bioreactor volume is typically harvested every two to four days and replaced with fresh culture medium (IIC:1.5.2c:2). Conditioned medium from one or more bioreactors is purified to create one batch of rFIX drug substance. This culture process has been run continuously for up to 58 cell population doublings from the WCB (~17 batches of drug substance), over a period of approximately two months from a single inoculum (IIC:1.5.2g). Cell generations are tracked for each bioreactor, and based on maximal cumulative population doublings used to date, Genetics Institute has appropriately imposed within each inoculum run a process limit (Table IIC:1.5.2f:1.2-1) of 58 generations from the WCB for cells from any bioreactor harvested to make a batch of drug substance. Material made throughout the course of an inoculum run is comparable by all analytical tests, except possibly for a slight trend (up to 10%) towards increasing specific activity (IIC:1.10).

Other in-process cell culture monitoring for each bioreactor harvested includes cell viability, cell density, cell doubling time, cellular productivity for rFIX, pH, temperature, dissolved oxygen, bioburden and mycoplasma. In addition, for each inoculum run there is post-production testing (on end-of-production cells, not on each bioreactor harvest) for bacterial, mycoplasmal, fungal and viral contamination (Table IIC:1.5.2f:1.2-1). This in-process monitoring is conventional and in compliance with the expectations of regulatory guidelines (ICH Draft Guideline on Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin).

Purification. The purification of rFIX does not involve the use of a monoclonal antibody column and its attendant complications, such as the potential for contamination by antibody and murine viruses, or the need for denaturing elution conditions. Instead, the purification uses a series of four chromatography resins (Figure IIC:1.5.3c-1) and takes advantage of intrinsic properties of the rFIX molecule to adsorb and desorb the molecule under mild conditions. Each of the resins is standard to the pharmaceutical industry. The first chromatographic step is designed as a pseudo-affinity chromatography step to be highly selective. rFIX in diafiltered and concentrated conditioned medium is loaded on a quaternary amine resin (Q Sepharose Fast Flow) and the rFIX is subsequently eluted with a low concentration (10 mM) of calcium chloride (IIC:1.5.3c:1). The calcium ions induce a conformational change in rFIX that results in selective elution from the resin. Subsequent to the Q Sepharose step, there are three additional chromatography steps.

In an effort to provide extraordinary assurance to end users and their physicians who are worried about unknown and currently undetectable infectious agents, a virus-retaining filtration step (Viresolve™, molecular weight cutoff 70,000) which allows passage of rFIX has been incorporated into the process since the middle of the clinical trials. This has been accomplished with minimal loss of rFIX (Table IIC:1.5.3i:2-1) and no adverse effects on the resulting drug substance or drug product.

Process Validation. Consistent with ICH Guidelines, the rFIX production process is characterized by a multifaceted approach to viral safety, including (1) the control of raw materials, (2) analyzed purity of the cell banks, (3) rigorous post-process virus testing, (4) control of the manufacturing conditions, and (5) validation of the virus-removing capacity of the other purification steps (IIV). The virus-retaining filtration step provides approximately 10^5 -fold removal of appropriately diverse model viruses (amphotropic murine leukemia virus, bovine parvovirus, human herpes simplex virus type I, parainfluenza virus, reovirus type 3) (Table IIC:1.8b:2-1). In addition to the virus-retaining filtration step, two other purification steps (Q Sepharose FF and Chelate-EMD-Cu^{++}) have been investigated for their ability to remove model viruses. The overall fold-removal from these three steps (a minimum of 10^{10}-fold for each model virus) is sufficient to provide assurance of removal of any adventitious viral contamination not otherwise detectable during post-process virus testing (Section IIV:4.1).

Other process validation studies include removal of host cell proteins (~10^4-fold; IIC:1.8b:3.2), PACE (~10^5-fold; IIC:1.8b:3.3), rhInsulin (>100-fold through the Q-Sepharose step alone; IIC:1.8b:4.6), methotrexate (>100-fold by the Q Sepharose step alone; IIC:1.8b:4.1) and DNA (>10^9-fold; IIC:1.8b:3.1) as well as many other process components (IIC:1.8). These studies have indicated that each of these potential contaminants is reproducibly removed to low, nonproblematic levels. The purification process has not been validated for the removal of proFIX; however, given the consistently low levels of proFIX in cell culture media (Figure IIC:1.8a:2.2-7), the validation of proFIX processing (IIC:1.8a:2.2), and the consistently low levels of proFIX measured in the drug substance (Figure IIC:1.8a:2.2-5), this omission is not problematic. As mentioned below, the removal of copper ions during the final ultrafiltration/diafiltration has been validated (IIC:1.8b:5.3).

Data from in-process monitoring indicate that the purification process has reproducible yields at each step (IIC:1.5.3i:2). In-process control of the purification process includes pH measurement of all buffers (IIC:1.5.4d). In addition to substantial manufacturing experience (4 inocula; 48 batches) at the one-bioreactor scale, 17 batches have been manufactured from one inoculum at a larger (2 and 3 bioreactor) scale. All in-process monitoring and drug substance release testing results have indicated that the manufacturing process is robust and reproducible (IIC:1.5.2g; IIC:1.5.3i; IIC:1.10).

Although the virus-retaining filters are single-use, the column chromatography resins and the microfiltration and ultrafiltration/diafiltration membranes are reused after cleaning with appropriate cleaning agents (i.e. NaOH or NaOCl). Having thus far seen no deterioration in resin or membrane performance (IIC:1.8d and IIC:1.8e), Genetics Institute has as yet placed no limitation on the number of times the resins and membranes may be reused. This is acceptable because there is an on-going program in place to assess the suitability of resin and membrane reuse and ultimate life.

Drug Substance Specifications and Release Testing. Drug substance release testing (Table IIC:1.1a:1-1) includes appropriately specific and sensitive tests for: purity (SDS-PAGE, RP-HPLC, SEC); safety (endotoxin, bioburden); potency (clotting assay); concentration (SEC); specific activity (activity/concentration); and identity (extent of gamma-carboxylation, peptide mapping, N-glycan carbohydrate fingerprinting, and SDS-PAGE). IEF analysis for carbohydrate charge distribution was not necessary due to the superior resolving power and structural information obtained from N-glycan carbohydrate fingerprinting.

Using standard electrophoretic and HPLC-based assays, specifications have been set which restrict the amount of the total detectable non-rFIX protein species to ≤1%. Results of testing to date have indicated very low levels (≤0.6%) of non-rFIX impurities (IIC:1.9b:6). The only potential non-rFIX protein contaminants that are possible for this manufacturing process are rhInsulin, PACE and host cell proteins. As mentioned above, validation studies have indicated that these proteins are consistently removed. Preclinical and clinical data have indicated that BeneFIX™ is well tolerated and have not indicated a need for further release specifications (see Preclinical and Clinical expert reports).

Genetics Institute has chosen to validate the removal of DNA and host cell proteins (IIC:1.8b:3), rather than implement specific routine release assays. The DNA and host cell protein validations examined the historical norms for the amounts of these materials in conditioned media from rFIX manufacture and found them to be consistent. In addition, drug substance purity measurements (SDS-PAGE and HPLC) have been examined for their ability to detect host cell proteins at levels which would indicate failure of the manufacturing process. This approach is not typical of industry practice, but is appropriate given the growing consensus that neither DNA nor host cell proteins represent a safety risk (FDA Workshop on Characterization of Biotechnology Pharmaceutical Products, Washington, D.C. December, 1995).

Similarly, Genetics Institute has chosen to validate that PACE will be removed during the purification process (IIC:1.8b:3.3) rather than employ a routine release assay. Given the extent and reproducibility of the manufacturing process data to date, the implementation of specific drug substance release assays for PACE is not needed.

The final chromatography column contains copper ions. Genetics Institute has performed concurrent validation studies which demonstrate that the diafiltration step will remove excess copper ions >100-fold. No drug substance assay or specification for copper is proposed. To date, copper ions have been detected (limit of detection 0.01 μg/mL) in 33 of 65 drug substance batches, and 11 of these 33 contained quantifiable levels (> 0.05 μg/mL) of up to 0.29 μg/mL (Table IIC:1.9b:6-1). The copper levels in these batches have not been observed to be problematic to the specific activity (Table IIC:1.10b-3) of the drug substance.

Preclinical and clinical investigations have indicated that the in vivo recovery of BeneFIX™ is consistently lower than Mononine™. Genetics Institute has been able to attribute this lower recovery to a lack of phosphorylation and a relative lack of sulfation of the recombinant molecule, and have established lot-to-lot consistency of recovery of rFIX in both an animal model and in human clinical trials (see Preclinical and Clinical Expert Reports). Because the maximum possible effect on recovery

is slight and because the lot-to-lot consistency of BeneFIX™ has been established, Genetics Institute is justified in not establishing release assays for sulfation, phosphorylation, or in vivo recovery.

Characterization of the rFIX Molecule. Extensive characterization of the rFIX drug substance (IIC:1.6) has indicated that although it is comparable to pdFIX, the rFIX molecule is not identical to the pdFIX molecule. As described above, the primary amino acid sequences of the two molecules are identical, although there is allelic heterogeneity in the pdFIX molecule at the Ala/Thr^{148} site. Both molecules are β-hydroxylated (~48% for rFIX; ~37% for pdFIX) at Asp^{64} (in the first EGF domain) and both are sulfated at Tyr^{155} (in the activation peptide), although pdFIX is sulfated to a greater extent (>90%) at this position than rFIX (~25%) (Figure IIC:1.6h:3.2-2).

The primary differences between the rFIX and pdFIX are that: the rFIX molecule is not phosphorylated at Ser^{158} (in the activation peptide), while the pdFIX is substantially phosphorylated; the pdFIX molecule appears to have more heterogeneity in N-linked carbohydrate structures than the rFIX molecule; pdFIX is fully gamma-carboxylated at all 12 sites, whereas rFIX is somewhat under-gamma-carboxylated at two of the twelve sites (mostly at Glu^{40} and to a lesser extent at Glu$^{36)}$. None of these differences appears to affect the in vitro clotting activity of the rFIX molecule (IIC:1.6h), nor is there an effect on efficacy or on the half life in patient plasma (see Clinical expert report). The recoveries of pdFIX and rFIX in patient plasma are both lower than the recoveries observed for factor VIII (~100%), with the recovery of rFIX (~38%) being less than that observed for Mononine (~51%) (see Clinical Expert Report). As a result of extensive investigations of these phenomena, these differences in recovery have been attributed primarily to the differences in sulfation and phosphorylation of the two molecules (see Preclinical Expert Report).

Higher order structure investigations in the presence and absence of calcium ions using circular dichroism (both near and far UV), tryptophan fluorescence, and sedimentation velocity ultracentrifugation have indicated that rFIX is indistinguishable from pdFIX from which high molecular weight species have been removed (IIC:1.6i).

The excipients used to formulate the finished product meet national and international compendial requirements and therefore have appropriate specifications and test methods (IIC:2.1.1).

IC:1.2.2.6 CONTROL TESTS ON INTERMEDIATE PRODUCTS

Not applicable.

IC:1.2.2.7 CONTROL TESTS ON THE FINISHED PRODUCT

Each lot of BeneFIX™ is filled on the basis of potency as measured in an in-process single-stage clotting assay (IIC:2.4b:2). Samples of each filled and lyophilized lot are then reconstituted and tested for potency using the single-stage clotting assay, and the lot is labeled on the basis of this potency assay. The chromogenic assay described in the European Pharmacopoeia is not used. After reconstitution, the results of the clotting assay and the determination of protein concentration (by SEC) allow a sufficiently accurate determination of the specific activity of each lot of rFIX to reasonably control the product potency.

Because lyophilization has the potential to form aggregates in protein preparations, each lot of BeneFIX™ is assayed for high molecular weight (HMW) material by an SEC assay. Although Genetics Institute has shown that lots of pdFIX have exhibited amounts of inactive aggregates in the range of 10 to 20% of total protein (IIC:1.6i:1), the manufacturing experience to date with BeneFIX™ (15 lots) has been reproducibly ≤1% (Table IIE:2.2c-2). Because HMW material has been observed at up to 1.4% for the drug substance, and given the potential for the lyophilization process and the storage of the lyophilized product to lead to aggregate formation, the high molecular weight species specification has been set at ≤4% for the drug product, versus ≤3% for the drug substance. As a consequence of the relative absence of HMW material in BeneFIX™ compared with Mononine™, the observed specific activity of the rFIX is correspondingly higher, ~260 IU/mg for BeneFIX™ versus ~220 IU/mg for Mononine™ (Table IIC:1.6i:1-1). As a further consequence, the specification for the specific activity of BeneFIX™ (200 to 360 IU/mg) has been established to be slightly higher than that for Mononine™, ≥150 IU/mg (U.S. FDA Summary of Basis for Approval).

To guard against degradation or contamination of rFIX during the filling process, the SDS-PAGE purity assay is used for each drug product lot, using the same specification for impurities (≤1%; Table IIE:1.1.1-1) as for the rFIX drug substance.

Additional specifications for appearance (before and after reconstitution), pH, residual moisture, particulate matter, activated FIX, bacterial endotoxin and sterility meet compendial expectations (Table IIE:1.1.1-1). No specification for the General Safety Test (in the U.S.) or Abnormal Toxicity (in the E.C.) is set, as is appropriate for such a well controlled manufacturing process and such a well characterized molecule. Having tested the clinical lots of rFIX in both the Limulus Amoebocyte Lysate (LAL) assay for endotoxin as well as the pyrogen test in rabbits, the decision to test the marketed product solely by the validated LAL test is appropriately sparing of test animals.

There is no release test of the finished product such as isoelectric focussing that directly indicates the charge of the rFIX molecule. Monitoring of this parameter is indirectly accomplished at the drug substance stage using carbohydrate fingerprinting of N-linked carbohydrate (extent of sialylation) and ion-exchange resin elution profiles (Gla content). Stability studies (see below) have indicated that neither the lyophilization process nor storage of the drug substance or drug product under the recommended conditions appears to affect this parameter.

In general, the release tests and specifications for BeneFIX™ are consistent with the analytical validation results (see below), lot release data to date (Table IIE:2.2c-2), stability studies (see below), and regulatory guidelines (IIE:1.1.2).

Genetics Institute proposes to package individual vials of BeneFIX™ in a kit containing a vial of commercially available Sterile Water for Injection (for reconstitution of the BeneFIX™) and appropriate sterile withdrawal and infusion devices (IA:2.2).

IC:1.2.2.8 ANALYTICAL VALIDATION

As recommended in the ICH Guideline on Validation of Analytic Procedures, all release assays have been appropriately validated to be precise, reproducible, and sensitive, given the product specifications (IIE:2.1). The clotting activity assay has been developed using working reference

material composed of pooled normal plasma and calibrated against the NIBSC International Standard for Blood Coagulation Factor IX, Concentrate (IIE:1.2.1d). Other assays use an appropriately qualified rFIX reference material (IIC:1.7c:2). The effects of sample matrix on each assay have been investigated and are understood. These validation studies (IIE:2.1) indicate that the release assays are robust.

IC:1.2.2.9 STABILITY

Forced decomposition studies (IIC:1.6k) have been used to determine the potential degradation pathways for the rFIX molecule. These studies have shown that degradation products would have been detected in the real-time stability studies, had they occurred.

Using rFIX from the one-bioreactor process, the stability of the drug substance (3 lots at –80 °C) and the drug product (3 lots of 1000 IU/vial; 1 lot of 500 IU/vial; 3 lots of 250 IU/vial; all at 2 to 8 °C) have been investigated. Given that the formulation of the three dosage strengths is identical, the bracketing approach used for stability studies is consistent with the ICH guideline on stability testing.

Data to date (IIF) indicate that the drug substance is stable for at least 18 months at –80 °C (IIF:1e:1) and the drug product is stable for at least 18 months at 2 to 8 °C (IIF:2g:1). The lyophilized product is also stable for at least 6 months at room temperature (IIF:2f). Studies of the reconstituted material have indicated that it is stable for at least 24 hours at room temperature (IIF:2g:2).

Given the linear nature of the scale-up of the process and the biochemical comparability of the multiple bioreactor process material to the one bioreactor process material, setting the expiration dating for the drug substance at 18 months and for the drug product at 18 months based on the data from the one bioreactor process is appropriate and in conformance with ICH guidelines. Extension of this expiration dating will be appropriate when later data become available. Genetics Institute has committed to and has begun confirmatory studies that will make use of lots of drug substance and lots of drug product manufactured using the multiple bioreactor process (IIF:1g and IIF:2h). These confirmatory stability data are to be achieved by putting one lot of drug substance per year and one lot of drug product per year on the confirmatory testing program (IIF:2h).

The effects of freeze-thaw, light, shipping conditions and elevated temperature (40 °C) on BeneFIX™ have all been appropriately investigated. Studies have indicated (IIF:2c:2.5) that the only instability observed for the lyophilized rFIX product appears to be a slight tendency to form high molecular weight material (aggregates) at elevated temperature (<1% over 3 months at 40 °C). There is no evidence that aggregates form under the recommended storage conditions.

IC:1.2.2.10 EVALUATION CONCLUSIONS

Factor IX is a very complex, highly posttranslationally modified molecule. Although very similar, the rFIX produced by Genetics Institute is not identical to the corresponding pdFIX. Nonetheless, rFIX is potent, pure, and stable, and as such is appropriate for its intended use. Both animal data and human clinical trial data (see corresponding expert reports) further support this conclusion.

The manufacturing process for the active ingredient of rFIX is well defined, under good control, robust, and follows current good manufacturing practices for recombinant DNA-based products. Because the cell banks have been well characterized and all other raw materials are essentially chemically defined, there is extremely little chance of contamination of rFIX by infectious agents. By contrast, the manufacture of the plasma-derived material is dependant on the blood supply, with each lot stemming from tens of thousands of different donors, each of whom has the potential for introducing both detectable and undetectable blood-borne pathogens into the production system. By design, rFIX is thus significantly safer than pdFIX with regard to blood-borne pathogens. For the same reasons, it is also not surprising that rFIX is more homogeneous than pdFIX.

The tests chosen to control the manufacture of the active component and the final dosage form address the issues of purity, potency, identity, and safety. These tests have been appropriately validated and are appropriately controlled by the use of reference standards. The most critical test is the traditional clotting activity assay, in which the rFIX molecule must interact correctly with all components of the tenase complex (calcium ions, phospholipid, factor VIIIa, and factor X) to produce factor Xa. This activity assay is extremely relevant to the clinical use of the product, and the specific activity of the rFIX molecule in this assay is an excellent indicator that the rFIX molecule has the appropriate attributes for use in treating hemophilia B.

Using release testing, rFIX from the multiple bioreactor process is indistinguishable from rFIX from the earlier 1 bioreactor process that was used for most of the clinical trials (IIC:1.10b:1). Although rFIX demonstrates somewhat lower recoveries in patient plasma than Mononine, these recoveries are reproducible, consistent lot-to-lot and appropriate for the treatment of bleeding episodes (see Clinical and Preclinical Expert Reports).

The rFIX dosage form is appropriately stable for its intended use. The dosage form and strengths chosen are traditional to the hemophilia B population.

IC:1.2.3 REFERENCES

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IC:1.2.4 INFORMATION ON THE EXPERT

Frederick T. Gates, III Ph.D.

July 26th, 1996
Date

Dr. Gates received a B.S. degree in Molecular Biophysics and Biochemistry from Yale University in 1971, and a Ph.D. in Biochemistry from the University of California at Berkeley, in 1976. After postdoctoral studies at Rockefeller University and the National Institute for Allergy and Infectious Diseases of the NIH, in 1980 Dr. Gates joined what was then the Office of Biologics of the Food and Drug Administration as a Senior Staff Fellow. While at the FDA, Dr. Gates conducted research in the molecular biology of the immune system, coauthored a number of Points to Consider documents, reviewed Investigational New Drug and Product Licencing Applications, and inspected manufacturers of recombinant biological products.

In 1987 Dr. Gates (by then a Research Chemist with the Center for Biologics Evaluation and Research (CBER)) left the FDA and joined Genetics Institute as Head of Regulatory Affairs. From 1990 to 1994, he served as the industry representative to the FDA's Blood Products Advisory Committee. Currently, Dr. Gates is Director of Regulatory Affairs at Genetics Institute, and he has been instrumental in developing the Chemistry, Manufacturing and Control information for a number of Genetics Institute's products, including rAHF (Recombinate®), EPO (Recormon®/Epogin®), and rFIX (BeneFIX™).

A curriculum vitae for Dr. Gates follows.

July, 1996

CURRICULUM VITAE

Frederick T. Gates, III, Ph.D.

Date of Birth:	April 5, 1949	
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Marital Status:	Married, two sons	

Education:

1971	B.S., Molecular Biophysics and Biochemistry, Yale University
1976	Ph.D., Biochemistry, University of California, Berkeley

Appointments:

1992-present	Director, Regulatory Affairs, Genetics Institute, Inc. 87 CambridgePark Drive, Cambridge, MA 02140
1990-1994	Industry Representative, Blood Products Advisory Committee, U.S. Food and Drug Administration
1989-1992	Associate Director, Regulatory Affairs, Genetics Institute, Inc. 87 CambridgePark Drive, Cambridge, MA 02140
1987-1989	Head, Regulatory Affairs, Genetics Institute, Inc. 87 CambridgePark Drive, Cambridge, MA 02140
1983-1987	Research Chemist, Food and Drug Administration, Center for Drugs and Biologics, Office of Biologics Research and Review, Division of Biochemistry and Biophysics, Cell Biology Branch, Bethesda, MD 20892
1980-1983	Senior Staff Fellow, Food and Drug Administration, Bureau of Biologics, Division of Biochemistry and Biophysics, Bethesda, MD 20892

1977-1980	Staff Fellow, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Immunogenetics, Bethesda, MD 20892
1976-1977	Postdoctoral Fellow (NIH Fellowship) The Rockefeller University, New York
1971-1976	Ph.D. Candidate in Department of Biochemistry, University of California, Berkeley
1970	NSF Undergraduate Research Program, Yale University

Society Memberships:
Harvey Society
American Association of Immunologists
Regulatory Affairs Professional Society

Honors:

1966-1967	Dalton Prize in Chemistry
1971-1976	Predoctoral Trainee, NIH
1976-1977	Postdoctoral Fellow, NIH
1987	Commendable Service Award, FDA

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BeneFix™

COAGULATION FACTOR IX
(RECOMBINANT)

DESCRIPTION

BeneFix,™ Coagulation Factor IX (Recombinant), is a purified protein produced by recombinant DNA technology for use in therapy of factor IX deficiency, known as hemophilia B or Christmas disease. Coagulation Factor IX (Recombinant) is a glycoprotein with an approximate molecular mass of 55,000 Da consisting of 415 amino acids in a single chain. It has a primary amino acid sequence that is identical to the Ala^{148} allelic form of plasma-derived factor IX, and has structural and functional characteristics similar to those of endogenous factor IX.

BeneFix™ is produced by a genetically engineered Chinese hamster ovary (CHO) cell line that is extensively characterized and shown to be free of infectious agents. The stored cell banks are free of blood or plasma products. The CHO cell line secretes recombinant factor IX into a defined cell culture medium that does not contain any proteins derived from animal or human sources, and the recombinant factor IX is purified by a chromatography purification process that does not require a monoclonal antibody step and yields a high-purity, active product. A membrane filtration step that has the ability to retain molecules with apparent molecular weights >70,000 (such as large proteins and viral particles) is included for additional viral safety. BeneFix™ is predominantly a single component by SDS-polyacrylamide gel electrophoresis evaluation. The potency (in international units, I.U.) is determined using an in vitro one-stage clotting assay against the World Health Organization (WHO) International Standard for Factor IX concentrate. One international unit is the amount of factor IX activity present in 1 mL of pooled, normal human plasma. The specific activity of BeneFix™ is greater than or equal to 200 I.U. per milligram of protein. BeneFix™ is not derived from human blood and contains no preservatives or added animal or human components.

BeneFix™ is inherently free from the risk of transmission of human blood-borne pathogens such as HIV, hepatitis viruses, and parvovirus.

BeneFix™ is formulated as a sterile, nonpyrogenic, lyophilized powder preparation. BeneFix™ is intended for intravenous (IV) injection. It is available in single-dose vials containing the labeled amount of factor IX activity, expressed in international units (I.U.). Each vial contains nominally 250, 500, or 1000 I.U. of Coagulation Factor IX (Recombinant). After reconstitution of the lyophilized drug product, the concentrations of excipients in the 500 and 1000 I.U. dosage strengths are 10 mM L-histidine, 1% sucrose, 260 mM glycine, 0.005% polysorbate 80. The concentrations after reconstitution in the 250 I.U. dosage strength are half those of the other two dosage strengths. The 500 and 1000 I.U. dosage strengths are isotonic after reconstitution, and the 250 I.U. dosage strength has half the tonicity of the other two dosage strengths after reconstitution. All dosage strengths yield a clear, colorless solution upon reconstitution.

CLINICAL PHARMACOLOGY

Factor IX is activated by factor VII/tissue factor complex in the extrinsic coagulation pathway as well as by factor XIa in the intrinsic coagulation pathway. Activated factor IX, in combination with activated factor VIII, activates factor X. This results ultimately in the conversion of prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin, and a clot can be formed.

Factor IX is the specific clotting factor deficient in patients with hemophilia B and in patients with acquired factor IX deficiencies. The administration of BeneFix,™ Coagulation Factor IX (Recombinant), increases plasma levels of factor IX and can temporarily correct the coagulation defect in these patients.

After single intravenous (IV) doses of 50 I.U./kg of BeneFix™ in 36 patients, each given as a 10-minute infusion, the mean increase in circulating factor IX activity was 0.8 ± 0.2 I.U./dL per I.U./kg infused (ranged from 0.4 to 1.28) and the mean biologic half-life was 19.4 ± 5.4 hours (ranged from 11 to 36). The in vivo recovery using BeneFix™ was statistically significantly less (28% lower) than the recovery using a highly purified plasma-derived factor IX product. There was no significant difference in biological half-life. In subsequent evaluations at 6 and 12 months, the pharmacokinetic parameters were similar to the initial results.

In clinical studies of BeneFix™ involving a total of 64 patients (44 previously treated patients [PTPs], 11 previously untreated patients [PUPs], and the 9 patients participating only in the surgical study), more than 7 million I.U. were administered over a period of up to 18 months. This includes 57 HIV-negative and 7 HIV-positive patients. Forty-five patients were evaluated for efficacy, all of whom were treated successfully for bleeding episodes on an on-demand basis or for the prevention of bleeds. Bleeding episodes that were managed successfully include hemarthroses and bleeding in soft tissue and muscle.

Management of hemostasis was evaluated in the surgical setting. Thirteen surgical procedures have been performed in 12 patients, with a cumulative dose ranging from 10,000 to 348,000 I.U. The 10 major procedures were performed using a pulse replacement regimen (N=7) or a continuous infusion regimen (N=3), and included a liver transplantation, a hernia repair, six orthopedic surgeries, and two dental extractions. Circulatory factor IX levels targeted to restore and maintain hemostasis were achieved with both pulse replacement and continuous infusion regimens. Hemostasis was maintained throughout the surgical period, and there was no clinical evidence of thrombotic complications in any of these patients. In four patients for whom fibrinopeptide A and prothrombin fragment 1 + 2 were measured preinfusion, at 4 to 8 hours, and then daily up to 96 hours, there was no evidence of significant increase in coagulation activation. Data from two additional patients were judged to be not evaluable.

A study of BeneFix™ has been initiated in patients who had not been treated previously with plasma-derived factor IX concentrate (PUPs). In preliminary data, 11 of the 20 patients enrolled in the study received at least one infusion of BeneFix.™ These 11 patients received a total of 27,208 I.U. in 42 infusions. Thirty to 50 patients will

be enrolled and followed for up to 5 years to complete evaluation of BeneFix™ in this patient population for safety, efficacy, and immunogenicity.

A low-level inhibitor developed in 1 of 44 BeneFix™ patients who had previously received plasma-derived products. This patient had an extensive previous history of exposure to plasma-derived factor IX products, including a single subcutaneous exposure, without history of inhibitor development. Antibodies were detected in this patient after 9 months of treatment (39 exposure days) with BeneFix.™ This patient was able to continue treatment with BeneFix™ with no anamnestic rise in inhibitor or anaphylaxis.

Twelve days after a dose of BeneFix™ for a bleeding episode, one hepatitis C antibody positive patient developed a renal infarct. The relationship of the infarct to prior administration of BeneFix™ is uncertain but was judged to be unlikely by the investigator. The patient continued to be treated with BeneFix.™

INDICATIONS AND USAGE

BeneFix,™ Coagulation Factor IX (Recombinant), is indicated for the control and prevention of hemorrhagic episodes in patients with hemophilia B (congenital factor IX deficiency or Christmas disease), including control and prevention of bleeding in surgical settings.

BeneFix™ is not indicated for the treatment of other factor deficiencies (e.g., factors II, VII, and X), nor for the treatment of hemophilia A patients with inhibitors to factor VIII, nor for the reversal of coumarin-induced anticoagulation, nor for the treatment of bleeding due to low levels of liver-dependent coagulation factors.

CONTRAINDICATIONS

Because BeneFix,™ Coagulation Factor IX (Recombinant), is produced in a Chinese hamster ovary cell line, it may be contraindicated in patients with a known history of hypersensitivity to hamster protein.

WARNINGS

As with any intravenous protein product, allergic type hypersensitivity reactions are possible. Patients should be informed of the early signs of hypersensitivity reactions including hives, generalized urticaria, tightness of the chest, wheezing, hypotension, and anaphylaxis. Patients should be advised to discontinue use of the product and contact their physician if these symptoms occur.

Since the use of factor IX complex concentrates has historically been associated with the development of thromboembolic complications, the use of factor IX-containing products may be potentially hazardous in patients with signs of fibrinolysis and in patients with disseminated intravascular coagulation (DIC).

PRECAUTIONS

General

Historically, the administration of factor IX complex concentrates derived from human plasma, containing factors II, VII, IX and X, has been associated with the development of thromboembolic complications.[1] Although

BeneFix™ contains no coagulation factor other than factor IX, the potential risk of thrombosis and DIC observed with other products containing factor IX should be recognized. Because of the potential risk of thromboembolic complications, caution should be exercised when administering this product to patients with liver disease, to patients post-operatively, to neonates, or to patients at risk of thromboembolic phenomena or DIC. In each of these situations, the benefit of treatment with BeneFix™ should be weighed against the risk of these complications.

Activity-neutralizing antibodies (inhibitors) have been detected in patients receiving factor IX-containing products. As with all factor IX products, patients using BeneFix™ should be monitored for the development of factor IX inhibitors (see **Clinical Pharmacology**). It has been reported[2] that patients dosed with high-purity plasma-derived factor IX products who develop inhibitors to factor IX are at increased risk of anaphylaxis upon subsequent challenge with factor IX.

Dosing of BeneFix™ may differ from that of plasma-derived factor IX products (see **Clinical Pharmacology** and **Dosage and Administration**).

Carcinogenesis, Mutagenesis, Impairment of Fertility

BeneFix,™ Coagulation Factor IX (Recombinant), has been shown to be nonmutagenic in the Ames assay and nonclastogenic in a chromosomal aberrations assay. No investigations on carcinogenesis or impairment of fertility have been conducted.

Pregnancy Category C

Animal reproduction and lactation studies have not been conducted with BeneFix,™ Coagulation Factor IX (Recombinant). It is not known whether BeneFix™ can affect reproductive capacity or cause fetal harm when given to pregnant women. BeneFix™ should be administered to pregnant and lactating women only if clearly indicated.

Pediatric Use

Safety and efficacy studies are ongoing in previously treated children and adolescents and in previously untreated children (see **Clinical Pharmacology, Warnings,** and **Precautions**). During clinical studies conducted in PUPs, no adverse reactions related to therapy were reported in 42 infusions.

ADVERSE REACTIONS

As with the intravenous administration of any product, the following reactions may be observed after administration: headache, fever, chills, flushing, nausea, vomiting, lethargy, or other manifestations of allergic reactions. During clinical studies with BeneFix,™ Coagulation Factor IX (Recombinant), conducted in previously treated patients (PTPs), 60 mild adverse reactions definitely, probably, or possibly related to therapy were reported for 2548 infusions. These were nausea (16), discomfort at the IV site (13), altered taste (10), burning sensation in jaw and skull (6), allergic rhinitis (3), lightheadedness (2), headache (2), dizziness (1), chest tightness (1), fever (1), phlebitis/cellulitis at IV site (1), drowsiness (1),

Exhibit 2

dry cough/sneeze (1), rash (1), and a single hive (1). Twelve days after a dose of BeneFix™ for a bleeding episode, one hepatitis C antibody positive patient developed a renal infarct. The relationship of the infarct to prior administration of BeneFix™ is uncertain but was judged to be unlikely by the investigator. The patient continued to be treated with BeneFix.™

If any adverse reaction takes place that is thought to be related to the administration of BeneFix,™ the rate of infusion should be decreased or the infusion stopped.

DOSAGE AND ADMINISTRATION

Treatment with BeneFix,™ Coagulation Factor IX (Recombinant), should be initiated under the supervision of a physician experienced in the treatment of hemophilia B.

Dosage and duration of treatment for all factor IX products depend on the severity of the factor IX deficiency, the location and extent of bleeding, and the patient's clinical condition, age and recovery of factor IX. For all these reasons, doses administered should be titrated to the patient's clinical response and, when clinically indicated, factor IX activity recovery levels.

In an eleven patient crossover, randomized PK evaluation of BeneFix™ and a single lot of high-purity plasma-derived factor IX, the recovery was lower for BeneFix™ (see **Clinical Pharmacology**). On average, one international unit of BeneFix™ per kilogram of body weight increased the circulating activity of factor IX by 0.8 I.U./dL. In clinical efficacy studies, patients were initially administered the same dose of BeneFix™ as they had been previously treated with plasma-derived factor IX and their dosage was titrated upward if necessary to achieve the desired clinical response.

Dosing should be based on the reported clinical trial pharmacokinetic results for BeneFix.™ The following formula provides a guide to empirical dosage calculations:

number of factor IX I.U. required = body weight (in kg) x desired factor IX increase (%) x 1.2 I.U./kg

In the presence of an inhibitor, higher doses may be required.

The following chart[3] may be used to guide dosing in bleeding episodes and surgery:

Type of Hemorrhage	Circulating Factor IX Activity Required (%)	Frequency of Doses (h)	Duration of Therapy (d)
Minor			
Uncomplicated hemarthroses, superficial muscle, or soft tissue	20-30	12-24	1-2
Moderate			
Intramuscle or soft tissue with dissection, mucous membranes, dental extractions, or hematuria	25-50	12-24	Treat until bleeding stops and healing begins; about 2 to 7 days
Major			
Pharynx, retropharynx, retroperitoneum, CNS, surgery	50-100	12-24	7-10

Source: Roberts and Eberst[3]

Alternatively, physicians with patients switching from plasma-derived factor IX to BeneFix™ may recommend an initial dose the same as the dose previously used for plasma-derived factor IX and titrate upward if necessary to achieve the desired clinical response.

For surgical interventions in particular, precise monitoring of the factor IX replacement therapy using the factor IX activity assay is advised.

BeneFix™ is administered by IV infusion over several minutes after reconstitution of the lyophilized powder with Sterile Water for Injection (USP).

If any adverse reaction takes place that is thought to be related to the administration of BeneFix,™ the rate of infusion should be decreased or the infusion stopped.

INSTRUCTIONS FOR USE

Reconstitution

Always wash your hands before performing the following procedures. Aseptic technique should be used during the reconstitution procedure.

BeneFix,™ Coagulation Factor IX (Recombinant), will be administered by intravenous (IV) infusion after reconstitution with Sterile Water for Injection (diluent).

1. Allow the vials of lyophilized BeneFix™ and diluent to reach room temperature.
2. Remove the plastic flip-top caps from the BeneFix™ vial and the diluent vial to expose the central portions of the rubber stoppers.
3. Wipe the tops of both vials with the alcohol swab provided, or use another antiseptic solution, and allow to dry.

4. Remove the protective cover from the short end of the sterile double-ended needle and insert the short end into the diluent vial at the center of the stopper.
5. Remove the protective cover from the long end of the needle and insert the long end into the BeneFix™ vial at the center of the stopper.

   Note: Point the double-ended needle toward the wall of the BeneFix™ vial to prevent excessive foaming.
6. Fully invert the diluent vial to the vertical position and allow the diluent to run completely into the BeneFix™ vial.
7. Once the transfer is complete, remove the long end of the needle from the BeneFix™ vial, recap, and properly discard the needle with the diluent vial.

   Note: If the diluent does not transfer completely into the BeneFix™ vial, DO NOT USE the contents of the vial. Note that it is acceptable for a small amount of fluid to remain in the diluent vial after transfer.
8. Gently rotate the vial to dissolve the powder.
9. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Reconstituted BeneFix™ should appear clear and colorless.

BeneFix™ should be administered within 3 hours after reconstitution. The reconstituted solution may be stored at room temperature prior to administration.

Administration (Intravenous Injection)

BeneFix,™ Coagulation Factor IX (Recombinant), should be administered using a single sterile disposable plastic syringe. In addition, the solution should be withdrawn from the vial using the sterile filter spike.

1. Using aseptic technique, attach the sterile filter spike to the sterile disposable syringe.

   Note: Do NOT inject air into the BeneFix™ vial. This may cause partial loss of product.
2. Insert the filter spike end into the stopper of the BeneFix™ vial.
3. Invert the vial and withdraw the reconstituted solution into the syringe.
4. Remove and discard the filter spike.

   Note: If you use more than one vial of BeneFix,™ the contents of multiple vials may be drawn into the same syringe through a separate, unused filter spike.
5. Attach the syringe to the Luer end of the infusion set tubing and perform venipuncture as instructed by your physician.

After reconstitution, BeneFix™ should be injected intravenously over several minutes. The rate of administration should be determined by the patient's comfort level (see **Adverse Reactions**).

Dispose of all unused solution, empty vials, and used needles and syringes in an appropriate container for throwing away waste that might hurt others if not handled properly.

Storage

Product as packaged for sale: BeneFix,™ Coagulation Factor IX (Recombinant), should be stored under refrigeration at a temperature of 2 to 8°C (36 to 46°F). BeneFix™ may also be stored at room temperature not to exceed 25°C (77°F) for up to 6 months. Freezing should be avoided to prevent damage to the diluent vial. Do not use BeneFix™ after the expiry date on the label.

Product after reconstitution: The product does not contain a preservative and should be used within 3 hours.

How Supplied

BeneFix,™ Coagulation Factor IX (Recombinant), is supplied in single dose vials which contain nominally 250, 500, or 1000 I.U. per vial (NDC # 58394-003-01, 58394-002-01, and 58394-001-01, respectively) with diluent, double-ended needle for reconstitution, filter spike for withdrawal, infusion set, and two (2) alcohol swabs. Actual factor IX activity in I.U. is stated on the label of each vial.

REFERENCES

1. Lusher JM. Thrombogenicity associated with factor IX complex concentrates. *Semin Hematol.* 1991;28 (3 Suppl. 6):3-5.
2. Shapiro AD, Ragni MV, Lusher JM, et al. Safety and efficacy of monoclonal antibody purified factor IX concentrate in previously untreated patients with hemophilia B. *Thromb Haemost.* 1996;75(1):30-35.
3. Roberts HR, Eberst ME. Current management of hemophilia B. *Hematol Oncol Clin North Am.* 1993; 7(6):1269-1280.

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